Final Report



AQUAFIN CRC PROJECT 1B5: FEED TECHNOLOGY FOR TEMPERATE FISH SPECIES VOLUME 1: FEEDING STRATEGIES

D. Stewart Fielder, Debra Ballagh, Mark A. Booth, Patricia M. Pankhurst, Clint E. Becker & Geoff L. Allan





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NON-TECHNICAL SUMMARY

2001/208 Aquafin CRC – Feed Technology Temperature Fish Species: Volume 1: Feeding Strategies

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OVERALL OBJECTIVES:

- 1. To reduce costs of fingerling production.
- 2. To improve the cost-effectiveness of grow-out diets.
- 3. To validate improved feeds and feeding practices on a commercial scale.

Specific strategies for Volume 1: Feeding Strategies:

- 1. To determine the best combination of live feeds/inert feeds for larval mulloway.
- To determine the best weaning and feeding strategies for juvenile mulloway. (Results of research on similar strategies for yellowtail kingfish will be reported at the conclusion of the project in July 2009).

The report is presented in two volumes Aquafin CRC – Feed Technology Temperature Fish Species: Volume 1: Feeding Strategies and Volume 2: Diet Development. The volumes share common background, need, overall objectives, benefits and adoption, further development, planned outcomes, intellectual property and staff. They have individual non-technical summaries, results and discussions and conclusions.

NON TECHNICAL SUMMARY:

Introduction

Mulloway (*Argyrosomus japonicus*) were first bred in Australia by the team at Port Stephens Fisheries Institute in NSW in 1992. This species has attracted considerable aquaculture potential due to its almost Australia-wide distribution and fast growth rates. Commercial grow-out of this species is occurring in New South Wales and South Australia. There was zero production in 2000/01 and combined production in 2001/02 was only 46 t. Production of mulloway increased quickly to over 600 t per annum in 2006/07 (although this dropped off to 309 in 2007/08) and yellowtail kingfish (*Seriola lalandi*) increased to 3,370 t per annum (2007/08). Interest in mulloway has declined in favour of yellowtail kingfish. One notable difference between mulloway and kingfish is their feeding behaviour. Kingfish feed actively on the surface while mulloway are sub-surface feeders. Changing buoyancy of feeds is possible using extrusion technology but can restrict ingredient choice.

Temperate marine fish farmers in Australia seek to have the option to choose between several species of fish based on market price, availability and cost of fingerlings, health and feeding costs. This need was identified at the Aquafin CRC workshop held in 2002. At that time, most commercial and research interest on temperate marine finfish was with snapper (*Pagrus auratus*) but farmers moved quickly to yellowtail kingfish and mulloway. Applied research is needed, particularly on the major cost areas of diets and feeding (for both fingerlings and grow-out fish) to help ensure profitability and to give farmers and feed manufacturers information so they can make informed business decisions.

In hatcheries, the global shortage of Artemia and the huge cost of weaning diets led to a increased priority for better and cheaper live feeds, formulated weaning diets and feeding strategies. This project was designed to extend the successful research approach adopted for snapper in Aquafin CRC Project 1B.3-2001/208 (Increasing the profitability of snapper farming by improving hatchery practices and diets). In that project, fingerling costs were reduced by approximately 30% through systematic research to develop more cost-effective hatchery procedures including the demonstration of the feasibility of replacing live feeds including Artemia with alternative live feeds (copepods) and/or commercially available, inert pellet diets for advanced snapper larvae. Previous work with snapper also demonstrated a major improvement in growth of juvenile snapper when the optimal feeding frequency and day-length were identified. This project reduced feed costs, optimised feeding efficiency and improved fingerling survival and growth of mulloway. Similar research with yellowtail kingfish was also conducted. Sub-optimal performance of marine fish larvae is often a result of inadequate nutrition or sub-optimal physico-chemical variables during larval rearing. A high percentage of slow-growing or stunted fish in larval rearing runs can seriously reduce economic viability of hatcheries and increase farming costs. The performance of larvae has not previously been addressed in a systematic manner. Results from the research discussed in this report have been used to develop practical hatchery manuals for mulloway and vellowtail kingfish.

Embryonic development of mulloway (Argyrosomus japonicus) and egg surface disinfection using ozone

The embryonic development of mulloway was described to use as a visual reference of egg stages for use in mulloway hatcheries and egg disinfection protocols. Then, the effect of ozone disinfection on mulloway eggs was investigated. The first of two experiments examined the most appropriate CT (concentration [mg/l] x time [min]) value and temperature for disinfecting mulloway eggs with ozone, and the second experiment investigated the effects of ozone on the hatching success of larval mulloway at four different stages of embryonic development. Mulloway eggs begin to hatch 28 h post fertilisation at 22°C and eggs should be disinfected with a CT value of 1 at 22°C. Mulloway eggs can be treated at any stage of development and therefore should be disinfected with ozone early in development to reduce negative impacts of nodavirus and other pathogens on embryonic development.

Photoperiod and light intensity requirements of larval mulloway (Argyrosomus japonicus)

To optimise photoperiod and light intensity for mulloway, experiments were conducted to determine the effects of a range of photoperiods (24L:0D; 18L:6D; 12L:12D; 6L:18D and 0L:24D) and light intensities (1 and 10 μ mol s⁻¹ m⁻²) on the growth, swim bladder inflation, food consumption and survival of early (2-8 dah [days after hatching]) and advanced (14-34 dah) mulloway larvae. The two light intensities tested had no effect on the parameters measured and there were no significant interactions between photoperiod and light intensity. In early larvae, length was significantly improved (P<0.05) in fish held in 12, 18 and 24 h of light compared with fish in 6 and 0 h of light. Survival was significantly (P<0.05) reduced in 0 and 6 h of light compared with fish in 12, 18 and 24 h of light. Swim bladder inflation was significantly higher (P<0.05) in 6 h of light; however, there were no significant differences between swim bladder inflation of fish in 12 h of light compared with any other photoperiod.

In advanced larvae there were no effects observed in growth at the end of the experiment. Survival was significantly greater in the 0L:24D photoperiod compared with all other photoperiods. It is recommended that larvae are kept in a 12L:12D photoperiod until swim bladder inflation occurs followed by a 18L:6D photoperiod to promote growth

Weaning requirements of larval mulloway, Argyrosomus japonicus

Three experiments were conducted to examine if mulloway weaning practices can be improved by decreasing the amount of *Artemia* required during the weaning process to reduce the costs of production. This study determined that mulloway larvae can be weaned directly from rotifers to a pellet microdiet without the use of *Artemia*; however, this significantly reduces growth. Fish fed *Artemia* in combination with pellets also weaned successfully and displayed better growth rates, therefore the weaning process may be achieved more cost effectively by reducing the amount of *Artemia* used. Mulloway larvae began consuming *Artemia* in preference to rotifers once the mean length of larvae was 5.2 ± 0.5 mm TL. Mulloway larvae began consuming pellets in preference to *Artemia* once the mean length of larvae was 10.6 ± 1.8 mm TL.

Photoperiod and feeding interval requirements of juvenile mulloway, Argyrosomus japonicus

Two experiments were completed to determine the most efficient feeding interval and photoperiod for rearing juvenile mulloway, *Argyrosomus japonicus*. The feeding intervals were one feed every 1, 3, 6, 12 or 24 h and the photoperiods were 12L:12D, 18L:6D and 24L:0D. No interactions were found for any variables measured. The 12L:12D photoperiod produced significantly poorer survival than other photoperiods, however feeding interval did not influence survival. The 24L:0D photoperiod led to significantly reduced growth rates, compared to that of 12L:12D and 18L:6D. Fish fed once every 24 h had significantly reduced growth, however there was no difference in the growth of fish fed every 1, 3, 6 or 12 h. Juvenile mulloway should be reared in an 18L:6D photoperiod to promote growth and survival. Fish do not need to be fed more than twice a day or once every 12 h.

Changes in feeding behaviour and development of the sensory organs of larval and juvenile mulloway, *Argyrosomus japonicus*

The sensory organs (vision, mechanoreception or chemoreception) employed for feeding by larval and juvenile mulloway are likely to change as fish develop and grow. An experiment was conducted to determine the feeding modalities employed by larval and juvenile mulloway as ontogeny proceeds and was designed as a multi-factor experiment, in which larval and juvenile mulloway were fed in light and dark conditions to determine the role of vision in feeding, while ablation of the neuromasts using streptomycin sulphate enabled the role of mecanoreception and chemoreception in non-visual feeding to be determined. Mulloway larvae rely on vision for feeding in early development stages. Once larvae reach 5 mm, the role of mechanoreception becomes increasingly important. Mulloway used vision for feeding when they were initially fed pellets; however, the role of mechanoreception again became important once the mean length was 22.5 mm.

The effect of temperature and salinity on the growth, feeding efficiency and survival of juvenile mulloway, *Argyrosomus japonicus*

The effects of temperature and salinity on the performance of juvenile mulloway, held in 100-1 experimental tanks were investigated. Performance of juvenile mulloway (~ 9.4g/fish) was assessed at four temperature treatments (17, 21, 26, and 31°C) over 28 days. Feed consumption increased with temperature while growth increased with temperature up to 26°C and then declined at 31°C. The food conversion ratios (FCR's) were best at 21 and 26°C and significantly lower than that at 31° C (0.60-0.62 compared to 0.84). The above demonstrated that the optimal temperature for growth and survival was 26°C, while the optimal temperature for FCR and condition is 21°C. It is therefore recommended that juvenile mulloway be reared in water temperatures ranging from 21- 26° C. In the second experiment, performance was assessed after transfer from 34‰ to four salinity treatments (5, 15, 25, and 34‰) (water temperature $\approx 26^{\circ}$ C) for juvenile mulloway (~ 26.4g/fish) over 14 days. Survival was high and not affected by salinity but feed consumption, growth and FCR were better at 15 and 25‰. The results indicate that growth and feeding efficiency of juvenile mulloway may be improved by rearing them at intermediate salinities, when water temperatures are optimal (i.e. 26°C). In the third experiment, performance was assessed at combinations of two temperatures (21 and 26°C) and two salinities (5 and 34‰) in juvenile mulloway (~ 15.2g/fish) over 14 days. Feed consumption was not affected by salinity but increased significantly as temperature increased. Growth was significantly higher at 26°C than exposed 21°C. FCR and survival did not differ significantly. There were no interactive effects of temperature and salinity on any of the performance indicators.

Salinity and starter diets for juvenile yellowtail kingfish, Seriola lalandi.

The interactive effects of salinity (15, 22.5 or 30 ‰) and feed type (Ridley, Australian-made or Biomar Optimal starter, Denmark) on growth and feeding were investigated to guide decisions about sites for hatcheries, salinity management and choice of starter diets. For fish cultured in tanks for 14 days, there was no effect of salinity on growth or FCR but fish fed Biomar pellets were 30% heavier and FCR was 20% better than fish fed Ridley diet. There was no interaction between salinity and diet type. Sites with access to variable salinity seawater including estuaries may be suitable for land-based nursery or growout operations for yellowtail kingfish (YTK).

Fish size and feeding frequency for yellowtail kingfish, Seriola lalandi.

The interactive effects on growth and feeding of fish size $(3.0 \pm 0.03 \text{ g}, \text{small})$; or $96.0 \pm 1.0 \text{ g}, \text{large})$ and feeding frequency (1, 2 or 4 times/d or continual) between 0900 and 1500h was investigated to guide feed management strategies for juvenile yellowtail kingfish. Growth and FCR was affected by initial fish size and feeding frequency but there was no interaction. The average specific growth rate of small kingfish ($0.1\pm0.01 \text{ g/d}$) was 30% lower than that of large juvenile kingfish ($0.15\pm0.001 \text{ g/d}$) however FCR of small fish (range 0.96-1.0) was significantly lower than that of large kingfish (range 1.1-1.3). For small kingfish, growth was greatest when feed was delivered 4 times/d. After 35 d, small fish fed 4 times/d were 40% heavier than small fish fed 1 time/d. In contrast, feeding frequency had no effect on growth of large juvenile kingfish at any sampling time during the experiment.

Preliminary investigation into extensive culture of yellowtail kingfish, *Seriola lalandi*, larvae in fertilised ponds was unsuccessful

Most marine fish larvae species are cultured using indoor, intensive techniques. This method is expensive and requires dedicated, sophisticated facilities and skilled labour. Alternatively many freshwater and a small number of marine finfish including barramundi (Lates calcarifer), red drum (Sciaenops ocellatus), Australian snapper (Pagrus auratus) and mulloway (Argyrosomus *japonicus*) have been reared successfully using extensive methods in fertilised ponds relying on production of natural zooplankton in ponds filled with seawater and then fertilised to promote production of successive phyto- and zooplankton populations. Extensive rearing of YTK has not previously been attempted. Two attempts were made to evaluate if first-feeding YTK larvae could be cultured in ponds. Two, $500m^2$ plastic-lined ponds were 2/3 filled with 500 μ m filtered seawater and then completely filled with seawater from a production pond which had been fertilised with organic and inorganic fertilisers to promote phyto- and zooplankton production. Approximately 25,000 larvae (5 days after hatch) were stocked into two, 100-l acclimation tanks located next to each pond and were filled initially with seawater from the stock tank. Ponds were managed to maintain phyto- and zooplankton blooms for three weeks, at which time ponds were drained to catch any surviving juvenile kingfish. Neither experiment was successful. The most likely reason the first experiment failed was because pond temperatures fell to 16°C. Although YTK spawn in tanks at PSFI at 16°C, egg quality is poor and no eggs have ever developed and hatched. The reasons for total mortality of the kingfish larvae during the second experiment are unknown however post-handling mortality of early stage larvae is possible and may explain why no larvae were sampled during the culture period despite using rigorous netting techniques which are successful with other fish species including snapper and mulloway. The water quality parameters of outdoor ponds also tend to be more variable than those of intensive hatchery tanks and kingfish larvae may be susceptible to a changing environment. Further investigation should be done to determine if stocking advanced YTK larvae into ponds is more appropriate for production of juvenile YTK.

KEYWORDS:

Mulloway; Yellowtail kingfish; Feeding Strategies; Larval rearing; Ozonation; Photoperiod

1. BACKGROUND

Farming of marine fish in Australia is continuing to develop and is principally based on seacage growout of tuna (*Thunnus maccoyii*) and yellowtail (*Seriola lalandi*) in South Australia and Atlantic salmon (*Salmo salar*) in Tasmania. Production of yellowtail in SA was reported as 50 and 700 t in 2000/01 and 2001/02 respectively, and reached 1500 t in 2002/03 (Hutchinson, 2003). Production of mulloway (*Argyrosomus japonicus*) in South Australia has recently increased as farmers have sought an alternative species to culture following problems with yellowtail culture (the major problems include skin and gill flukes). In NSW, snapper (*Pagrus auratus*) mulloway, and silver bream (*Acanthopagrus australis*) are also being farmed in small quantities for domestic consumption. Small numbers of other estuarine species, including black bream (*Acanthopagrus butcheri*) sand whiting (*Sillago ciliata*) Mahi mahi (*Coryphaena hippurus*) and eels (*Anguilla australis* and *A. reinhardtii*), have been produced intermittently for research and or commercial evaluation in various states. Estimated combined production for these species in Australia for 2002/03 is close to 140 t. A large marine fish farming venture in Morton Bay, Qld is seeking approval but facing considerable local opposition.

Industry interest in temperate marine species is obviously driven primarily by economics but also by available technology, particularly for fingerling production and diets. Farmers in South Australia and NSW focused initially on snapper because hatchery technology was proven (although costs of fingerlings were very high) and attractive initial market prices. In SA, farmers turned from snapper to mulloway and then yellowtail following poor market prices for snapper (exacerbated by unattractive, dark skin colour) and relatively slow growth rates. Yellowtail was the species of choice in 2001 and 2002 because of rapid growth rates for this species and initial high market price. However, increasing problems with parasitic flukes, reducing market price and localised opposition to yellowtail farming encouraged the farmers to either shift back to mulloway or at least consider other species. The reduced market price for yellowtail was an inevitable consequence of increasing production but was also fuelled by the increasing value of the Australian dollar and the consequent reduction in export value and increasing competition from imports on the domestic market.

In NSW, farmers also started with snapper and although they also struggled with market acceptability due to skin colour, their proximity to the market made this easier to overcome. Availability of sufficient numbers of fingerlings restrained production and, at least for one farm, parasite problems were experienced. Mulloway and silver bream have been trailed as alternative species.

This shift between species is likely to remain a feature of Australian temperate marine fish culture for several reasons. Firstly, the current small demand has meant hatchery development has been slow and inherently risky. There are only a small number of hatcheries and this increases the risk that production of any particular species will fail. Having more than one species to breed, especially if natural breeding seasons are offset in time, reduces the risk of failure. Secondly, the small domestic market in Australia is particularly prone to supply-driven price decreases. Having several species available for sale mitigates this problem to some extent. Thirdly, at this stage in the industry, there is simply not enough industry experience to determine if one particular species is most suited to large-scale culture. Farmers still need the flexibility to experiment with a number of species to determine which ones suit their particular operation.

Technology for snapper has progressed rapidly following the Aquafin CRC project 1B.3-2001/208 (Increasing the profitability of snapper farming by improving hatchery practices and diets). When completed in 2007, this project will have significantly reduced both hatchery and diet costs. Two species currently have the most potential (and industry interest) as alternatives: yellowtail and mulloway. Yellowtail grows rapidly and is well suited to culture in sea cages. However, in SA,

high mortality rates have been experienced following infestations of the skin fluke (*Benedenia seriolae*) and the gill fluke (*Zeuxapata seriolae*). These organisms have also caused serious mortalities in Japan where approximately 100,000 t/yr of yellowtail are grown.

Although available "marine fish" diets have produced rapid growth rates, some farmers and feed manufacturers have questioned the nutritional adequacy of available diets, especially for rapidly growing fish. Slow growth, especially over winter, together with mortality unrelated to parasites, have also been attributed by some farmers to inadequate nutrition.

Mulloway were first bred in Australia by the team at Port Stephens Fisheries Institute in NSW in 1992. This species has considerable aquaculture potential due to its almost Australia-wide distribution and fast growth rates. Commercial grow-out of this species is occurring in NSW and SA but the combined production in 2002/03 was only several hundred tonnes. Interest in mulloway in southern states initially declined in favour of yellowtail but as mentioned above, has made a resurgence over the last year. Mulloway appears to be extremely hardy and very few problems with parasites or diseases have been recorded, despite quite extensive farming trials in NSW and SA. The potential for grow-out of this species is not limited to seacage culture. Due to its extremely euryhaline nature (5-35 ‰) the species has potential for estuarine pond culture and recent grow-out trials in both NSW and SA inland saline water bodies have been promising. The gregarious nature of this animal may allow culture at quite high densities that may allow the species to be cultured in recirculating aquaculture systems. One notable difference between mulloway and kingfish is their feeding behaviour. Kingfish feed actively on the surface while mulloway are sub-surface feeders. Changing buoyancy of feeds is possible using extrusion technology but can restrict ingredient choice.

Yellowfin bream (*Acanthopagrus australis*) are currently being grown by one farmer (Silver Beach Aquaculture) in Botany Bay who has indicated the fish are performing quite well and are exhibiting similar growth rates to the closely related snapper. Prices paid for bream are similar and can be better than that paid for snapper and fingerlings are available from northern NSW hatcheries.

INDUSTRY CONSULTATION

The research proposed in this project was listed as a priority area for research by the Aquafin CRC Joint Management Advisory Committee, the Yellowtail Kingfish Aquaculture Strategic R and D Plan 2003-2008 and at the Aquafin CRC Snapper Workshop (Allan 2003). It has been requested by marine fish farmers in NSW and SA and by the largest feed manufacturer in Australia (Ridley Pty Ltd). The priority placed on the research by industry is clearly demonstrated by an industry commitment of over \$235,610 in CASH to the research.

AQUAFIN CRC – PROJECT DEVELOPMENT AND PRIORITY

This project is the second of two projects I & I NSW Fisheries is leading under the Aquafin CRC. I & I NSW Fisheries signed up as a participant in the Aquafin CRC (2001-2008) to develop technology for temperate marine finfish farming. The first Aquafin CRC project "Increasing the profitability of snapper farming by improving hatchery practices and diets" (2001/029) was agreed to after extensive consultation with marine finfish farmers in NSW and SA. At the time the project was initiated, both major commercial farmers in NSW were collaborators and both are still participants of the Aquafin CRC. This second project has also been developed following extensive consultation with marine finfish farmers in NSW, Qld, WA and SA. A workshop was convened in Melbourne in September 2002 (Allan, 2003) where priorities for future research on temperate marine finfish were discussed. This project has arisen following these consultations. During the development phase for this project, individual marine fish farmers in SA and NSW, the South Australian Marine Finfish Farmers Association (SAMFFA), Ridley Aquafeeds Pty Ltd, the FRDC Aquaculture Nutrition Subprogram and, of course, all collaborating research providers were consulted. The Yellowtail Kingfish Aquaculture RandD Plan (2003-2008), lists as highest priority

fish health issues, and "next generation technologies" (objective 2 of this application addresses these issues), as high priority stress management (addressed by objective 2) and as medium priority nutrient impacts and carrying capacity (addressed by objective 2, particularly bioenergetic models). Strategic research issues to improve production efficiency and reduce costs were listed as "becoming increasingly important over time". The research in this application will build the foundation for these efficiencies and cost savings through improvement in grow-out diets and feeding technologies. Although initially very supportive, the SAMFA decided they were unable to commit cash to this project, partly because of the long-term nature of the project. Even so, outcomes from the project will be of significant benefit to SA marine fish farmers. Specifically, diets developed and adopted by the industry partner, Ridley Aquafeed Pty Ltd, will be available to SA farmers and SA farmers will be consulted about evaluation of project diets. Every effort will be made to involve them in feeding trials. Secondly, costs of fingerlings are very high in SA. Even though commercial farmers in SA do not list RandD to reduce fingerling production costs as a high priority, commercial fingerling costs are excessive (e.g. >\$2.00/fingerling for mulloway. This project will reduce costs of fingerling production. (As an example of previous success, costs for snapper fingerlings were reduced by 30% as a result of RandD on a previous Aquafin CRC project carried out by the current project team). The methodology described in this application is consistent with recommendations that have arisen from the Aquaculture Nutrition Subprogram workshops. This project was accorded priority from both CRC and FRDC boards (with suggested revisions in light of the SAMFA decision to reverse their support). The suggested revisions have all been made although budget is slightly larger (13.7%) than recommended by the FRDC Board.

2. NEED

Temperate marine fish farmers in Australia seek to have the option to choose between several species of fish based on market price, availability, cost of fingerlings and health and feeding costs. This need was identified at the Aquafin CRC workshop held in 2002 (Allan, 2003). Most research on temperate marine finfish has been conducted on snapper (*Pagrus auratus*) but farmers are now concentrating more on yellowtail (*Seriola lalandi*) and mulloway (*Argyrosomus japonicus*) with significant interest in yellowfin bream (*Acanthopagrus australis*). Applied research is needed, particularly on the major cost areas of diets and feeding (for both fingerlings and grow-out fish) to help ensure profitability and to give farmers and feed manufacturers information so they can make informed business decisions.

Costs of feeds and feeding are usually the largest budget expense for marine fish farms and also significantly affect costs of producing fingerlings in hatcheries. In hatcheries, the global shortage of Artemia and the huge cost of weaning diets has led to a increased priority for better and cheaper live feeds, formulated weaning diets and feeding strategies. For grow-out, most farmers want highperformance, low-cost feeds. Given a choice, most farmers will pay more for diets to achieve better performance but have no real way to make decisions to achieve the most cost effective feeding strategy. There is a clear lack of information for most temperate marine species about the nutritional specifications needed for high performance diets and what physical characteristics are most desirable in the pellets (e.g. should pellets be floating or sinking, how important is pellet hardness, etc). This prevents feed manufacturers providing data-based recommendations about the best diets for farmers and prevents them from formulating and manufacturing specific diets for temperate marine finfish farmed in Australia. Unfortunately, the same lack of information is restricting choices about the ingredients being used in diets. Almost no information exists about digestibility or utilization of most of the ingredients available for use in Australia aquafeeds. Most farmers are aware of "problems" with the use of terrestrial animal protein meals in animal feeds and that use of such ingredients might negatively affect the export market for their fish. However, apart from research with snapper (Aquafin CRC; WA Fisheries) and barramundi (FRDC ADD Subprogram; WA Fisheries) there is no information about digestibility or utilization of Australian ingredients for temperate marine finfish being farmed in Australia. The immediate result of this lack of information is an increase in the proportion of expensive, imported fishmeal being used in diets.

This project will extend the successful research approach adopted for snapper in Aquafin CRC Project 1B.3-2001/208 (Increasing the profitability of snapper farming by improving hatchery practices and diets). In that project, fingerling costs were reduced by approximately 30% through systematic research to develop more cost-effective hatchery procedures including the demonstration of the feasibility of replacing live feeds including Artemia with alternative live feeds (copepods) and/or commercially available, inert pellet diets for advanced snapper larvae. Previous work with snapper also demonstrated a major improvement in growth of juvenile snapper when the optimal feeding frequency and day-length were identified. This project seeks to reduce feed costs, to optimise feeding efficiency and to improve fingerling survival and growth of mulloway and vellowtail. Sub-optimal performance of marine fish larvae is often a result of inadequate nutrition or sub-optimal physio-chemical variables during larval rearing. A high percentage of slowgrowing or stunted fish in larval rearing runs can seriously reduce economic viability of hatcheries and increase farming costs. The performance of larvae has not been addressed in a systematic manner and although the commercial hatcheries in SA report that fingerling production is not a barrier, there are no published methods of how to optimize production of fingerlings (i.e. to to increase cost-effectiveness of fingerling production). This lack of information will reduce the chance of expanding marine fish farming in NSW and other states in Australia.

Existing grow-out diets used for marine fish such as yellowtail, mulloway and bream are based on generic formulations for "marine fish" (including salmon and barramundi). These diets produce results but it is unknown if current diets are nutritionally adequate, especially for rapidly growing fish. Even basic requirements, like the best protein to energy ratio, are unknown for yellowtail and mulloway. Both low and high energy diets are available for salmon and barramundi but even simple comparisons to find the best of these two "options" have not yet been carried out. There is no reliable information on ingredient digestibility making it impossible for feed manufacturers to confidently formulate diets with alternative protein sources to fishmeal when fishmeal is hard to obtain and when prices are high (and, of course, fish meal prices continue to rise). Research to provide this information is urgently needed.

There are obvious problems with a "one-species at a time" approach to diet development research. This is expensive and takes a long time. This application seeks to conduct specific research with mulloway and kingfish and to build comprehensive models of nutritional requirements for these two species that can be directly compared with other similar models now available for other marine and freshwater aquaculture species (e.g. snapper, sea bream and barramundi).

Fingerling costs for mulloway and kingfish are currently estimated at 0.60-200/fingerling. These represent well in excess of 10% of operating costs. We aim to reduce these costs by as much as 50%. Growout feeds can cost in excess of 2,000/t and with the costs of feeding are usually in excess of 30% of total operating costs (>50% for some operations). Food conversion ratios of in excess of 1.5:1 are regularly reported. We aim to produce diets with FCRs of 1.2:1 with approximately 25% lower ingredient costs. Together these represent the major areas where improvements in production technology can improve the profitability of marine fish farming.

3. OBJECTIVES

OVERALL OBJECTIVES:

- 1. To reduce costs of fingerling production.
- 2. To improve the cost-effectiveness of grow-out diets.
- 3. To validate improved feeds and feeding practices on a commercial scale.

Specific objectives for this Volume 1: Feeding Strategies:

- 1. To determine the best combination of live feeds/inert feeds for larval mulloway and yellowtail kingfish.
- 2. To determine the best weaning and feeding strategies for juvenile mulloway and yellowtail kingfish.
- 3. To determine the best weaning and feeding strategies for juvenile mulloway. (Results of research on similar strategies for yellowtail kingfish will be reported at the conclusion of the project in July 2009).

4. **RESULTS AND DISCUSSION**

4.1 Embryonic development of mulloway (*Argyrosomus japonicus*) and egg surface disinfection using ozone

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ABSTRACT

Mulloway, Argyrosomus japonicus, are a euryhaline sciaenid currently cultured at the Port Stephens Fisheries Institute (PSFI) in NSW, Australia. Culture techniques of this species are researched to improve production efficiency and to assist the industry to adapt to new challenges. In 2004, a nodavirus was identified at the PSFI and it was determined that management of viruses and bacteria should include egg disinfection. Consequently, it is now a requirement that all eggs are disinfected with ozone before they are transferred to the hatchery. In this study the embryonic development of mulloway was described to create a visual reference of egg stages for use in mulloway hatcheries and egg disinfection protocols. The first of two experiments investigated the combined effects of ozone CT (0, 0.1, 0.5, 1 or 5) and treatment temperature (19, 22 or 25°C) on the hatching success of mulloway larvae. The second experiment investigated the effect of ozone exposure (CT = 1) when applied at four different stages of embryonic development (3, 8, 20 or 27) hours post fertilisation; HPF) on the hatching success of larval mulloway. Hatching success for both experiments was measured by the percentage of larvae hatched, the time of hatching and the percentage deformity at hatching. In the first experiment, significantly fewer (P < 0.05) larvae hatched when the eggs were treated with an ozone CT of 5 compared with those treated with a CT of 0, 0.1, 0.5 or 1. In addition, significantly fewer larvae (P < 0.05) hatched when eggs were treated with an ozone CT of 5 at 19°C than when eggs were treated at 22 or 25°C. There were no apparent negative impacts of treating eggs at 22 or 25°C and as mulloway eggs are currently incubated at 22°C at the PSFI, it is recommended that 22°C remains the standard treatment and incubation temperature. The highest CT value that did not negatively affect hatching was a CT of 1, and therefore it is recommended that eggs should be ozone treated with a CT of 1 at 22°C. In the second experiment there was no significant effect (P>0.05) of ozone treatment on hatchability of eggs when applied at any of the development stages examined. Therefore, it is suggested that eggs should be disinfected with ozone early in development to reduce negative impacts on development caused by nodavirus and other pathogens.

1. INTRODUCTION

Mulloway, *Argyrosomus japonicus*, are a euryhaline sciaenid occurring along Australia's southern coastline. They are thought to spawn in coastal waters close to beaches (Silberschneider and Gray, 2008) and the buoyant eggs hatch 28-30 h after fertilization at 23°C (Battaglene and Talbot, 1994). Mulloway have been cultured in Australia for wild stock enhancement (Taylor et al., 2005) and for a growing aquaculture market (Battaglene and Talbot, 1994; Fielder et al., 1999). In New South Wales, mulloway are produced at the Port Stephens Fisheries Institute (PSFI) marine fish hatchery along with yellowtail kingfish (*Seriola lalandii*), snapper (*Pagrus auratus*) and Australian bass (*Macquaria novomaculata*). In 2004, a new strain of nodavirus was found in larvae of Australian bass cultured at the PSFI. The virus was identified as the Australian bass nodavirus (Peter

Kirkland, pers. comm. Elizabeth Macarthur Agricultural Institute), and it was determined that management of viruses and other pathogens should include an egg surface disinfection regime.

Nodaviruses are a family of neuropathogenic viruses capable of causing disease of the nervous tissue. They are a major pathogen of a wide range of larval and juvenile finfish and cause significant mortalities in aquaculture worldwide (Munday and Nakai, 1997). The disease caused by nodavirus has been named viral encephalopathy and retinopathy (VER) (Munday et al., 1992) and also Viral Nervous Necrosis (VNN) (Yoshikoshi and Inoue, 1990); VNN will be the term used in this manuscript. The disease is characterized by vacuolation and necrosis of the central nervous system and leads to a range of symptoms that include hyper-inflated swim bladders and atypical swimming, such as whirling, rotating and hyperactivity (Munday and Nakai, 1997; Grotmol et al., 1999).

Virus particles can be transferred horizontally to larvae after hatching, and it has been suggested that the entry point of infection occurs through the intestinal epithelium (Grotmol et al., 1999). Hatchery techniques usually employed to control horizontal transfer of nodavirus in marine hatcheries include strict hygiene protocols, sterilization of influent water and exposure of culture surfaces to high temperatures and pH (Frerichs, 2000). The virus can also be transferred vertically, where nodavirus particles present in broodstock ovaries are expelled during spawning (Munday and Nakai, 1997). The point of infection for vertical transfer may be through the connective tissue covering the oocytes, and can be most effectively controlled using egg surface disinfection by submersion of fertilised eggs in iodine or more commonly, ozone baths (Arimoto, 1996; Grotmol and Totland, 2000; Azad et al., 2005).

Ozone (O_3) is used for water treatment and disinfection in aquaculture because of its strong oxidation properties. Ozone in aquaculture is usually generated using corona discharge, which converts oxygen (O_2) to ozone (O_3) on contact (Summerfelt and Hochheimer, 1997). For egg disinfection the ozone gas is injected into a water bath in which the fertilised eggs are then submerged. The strength of an ozone bath is measured using a CT value, which is the concentration of ozone (mg/l) multiplied by the contact time (minutes). The oxidising properties of ozone are effective in inactivation of nodavirus and bacteria but can also be harmful to the eggs themselves and therefore, tolerance of eggs to ozone must be assessed on a species by species basis (Liltved et al., 1995; Grotmol et al., 2003).

A reduction in hatching ability has been reported as a common side effect of some ozone treatments (Hall et al., 1981; Grotmol and Totland, 2000; Buchan et al., 2006) and could be due to alterations made to the egg surface, including hardening, or a reduced secretion of hatching enzyme (Grotmol and Totland, 2000). It has been suggested that the tolerance of fish eggs to ozone may be correlated to the diameter of the egg (Ben-Atia et al., 2007), and also the temperature of incubation. When the eggs of gilthead sea bream (*Sparus aurata*) were treated with ozone at 19°C using CT values of 0.6 and 1.2, hatchability was not significantly affected, however when the CT value was increased to 2.4 and 4.8 hatching success was significantly reduced (Ben-Atia et al., 2007). In contrast, haddock (*Melanogrammus aeglefinus*) eggs that were incubated at 6°C were capable of tolerating ozone CT values up to 30 without survival decreasing (Buchan et al., 2006).

Only one case of VNN in mulloway larvae has been reported and consequently little is known of the biology of the virus and potential impacts to hatchery production. However, nodavirus has been previously detected in the larvae of another sciaenid, red drum, *Sciaenops ocellatus*, and significant mortality was observed from the resulting VNN (Oh et al., 2002). Nodavirus is therefore likely to cause similar problems for larvae production of mulloway if left uncontrolled or managed.

This study was designed to determine appropriate protocols for disinfecting mulloway eggs with ozone. The embryonic development of mulloway was described from fertilisation through to hatching to create a visual reference of egg stages for use in mulloway hatcheries and egg disinfection protocols. The first of two experiments were then designed to investigate the combined effects of ozone CT (0, 0.1, 0.5, 1 and 5) and treatment temperature (19, 22 or 25°C) on the

hatching success of mulloway larvae. The second experiment investigated the effect of ozone on the hatching success of larval mulloway at four different stages of embryonic development (3, 8, 20 or 27 h post fertilisation; HPF). Hatching success for both experiments was measured by the percentage of larvae hatched, the time of hatching and deformities at hatching.

2. METHODS

2.1 Mulloway embryonic development sequence

Mulloway broodstock were induced to spawn using Lutenising Hormone Releasing Hormone analogue (LHRHa) (50 μ g/kg). After fertilisation, a sample of eggs was transferred immediately to the laboratory where digital photographs were taken using a Motic Image Analyser (Extech Equipment Pty. Ltd., Boronia, Victoria, Australia). The remaining eggs were harvested from the tank and transferred to a holding bath maintained at 22°C. Photographs were taken of eggs from the holding bath at 45, 60, 75, 90 and 120 min after fertilisation and then every hour until hatching. The development of mulloway embryos at 22 °C was then documented using the photographs.

2.2 *Experiment 1 - The interactive effects of ozone CT and temperature on mulloway hatching success*

A factorial experiment was designed to determine the effect of ozone CT (CT = 0, 0.1, 0.5, 1 or 5) in combination with three temperatures (19 ± 0.1 , 22 ± 0.2 or 25 ± 0.2 °C). Fertilised mulloway eggs were obtained from a commercial mulloway hatchery (Clear Water Mulloway, Millers Forest, NSW, Australia). The eggs were divided into 45 groups, each with 120 eggs, and held in small size soft mesh aquarium nets to aid in transfer. Ozone was generated using an Ozotech ozone generator (Model - OZ1BTUS, California, USA) and was bubbled using a ceramic air stone into sea water held in 10 l buckets. The concentration of ozone was measured three times using a photometer (Model - ozone 1000, Palintest, UK) to ensure an accurate reading was obtained and the concentration of ozone remained stable. The concentration of ozone was adjusted to correspond with that of the desired CT value, therefore standardizing the treatment time for each replicate to 1 min. Each group of eggs was then treated individually with their respective ozone CT value and temperature at the gastrula stage (8-10 h post fertilisation; HPF) of development. Eggs were then transferred to 10 l hatching vessels, which were suspended in a water bath at the desired treatment temperature. The facilities available limited our ability to replicate hatching temperature; however the temperature in each experiment vessel was measured hourly to ensure consistency. The 101 treatment vessels were moderately aerated to maintain egg buoyancy for the duration of the experiment. The eggs were then observed and hatching milestones including time to start of hatching, 50 % hatched and the completion of hatching were recorded. Larvae and un-hatched eggs were then harvested and examined using a dissection microscope (M5 - 89734, Wild Heerbrugg,Switzerland) to provide total percentage of hatched larvae and larval deformities.

2.3 Experiment 2 - The effect of ozone treatment on hatching success for mulloway eggs treated at four stages of embryonic development

The effect of ozone on mulloway eggs treated at four embryonic development stages (3 HPF, cleavage; 8 HPF, gastrulation (early neurulation); 20 HPF, late development; and 27 HPF, larvae preparing to hatch) was investigated. Based on the results of Experiment 1, an ozone CT value of 1 was used, as this was the highest CT that did not negatively affect hatching.

Mulloway broodstock at the PSFI were induced to spawn using temperature cues (Partridge et al., 2003), and fertilized eggs were immediately collected and transferred to a holding tank which was maintained at $22 \pm 0.2^{\circ}$ C. Replicates of 100 eggs were counted into small size soft mesh nets approximately 15 min before the allocated treatment time. Each sampling time consisted of four ozone treated replicates and four control replicates. The control replicates were handled as for ozone treatments but were treated with an ozone CT of 0. Ozone was generated and measured as

described for Experiment 1. Eggs were treated for 1 min (1 or 0 mg/l ozone) and were then transferred to 100 l incubation tanks and maintained at 22 ± 0.3 °C in dark conditions with moderate aeration to maintain egg buoyancy. Eggs were monitored using torch light for the start of hatching, 50% hatched and the completion of hatching. Larvae and un-hatched eggs were then harvested and examined using a dissection microscope (M5 – 89734, Wild Heerbrugg, Switzerland) to provide total percentage of hatched larvae and larval deformities.

2.4 Statistical analysis

Statistical analyses were conducted using Statgraphics Version 4.1 (STSC, USA). In the first experiment, a two-factor analysis of variance (ANOVA) was used to determine the interactive effects of CT value and temperature on the percentage of hatched larvae. As the interactions between CT value and temperature were significant, data were compared using one way analysis of variance (ANOVA) separately for each temperature and each CT. As less than 50% of eggs in the 19°C and 5 CT treatment hatched, data for those treatments were not examined for hatching time (HPF 50% hatched) and duration of hatching. The data for Experiment 1 (percent hatched) was not normally distributed, and transformation failed to normalise the data. However, we are confident that ANOVA is robust enough to cope with these data (Underwood, 1981), and to reduce the risk of a Type1 error, significance levels were adjusted to $\alpha = 0.01$.

For the second experiment, means were compared using a two-factor ANOVA ($\alpha = 0.05$) for the percentage of larvae hatched, the hours (HPF) until hatching (50% of larvae) and the duration of hatching (hours from initial hatch to final hatch). Where statistical differences were found in analyses (*P*<0.05), the means were separated by the Student – Newman – Keuls test (SNK).

3. **RESULTS**

3.1 *Embryonic development sequence of mulloway*

Fertilised eggs (Fig. 1a) were positively buoyant, spherical in shape, transparent and contained one, sometimes two, oil globules. The first cell division (denoting the onset of cleavage) took place approximately 45 min post-fertilisation at the animal pole. The next three successive cleavages occurred every 15 min, but began to slow after the fourth division with the result that a clearly defined blastodermal cap (the blastodisc) was visible overlying the yolk mass, 4 HPF (Fig 1b). Between 4 and 6 HPF, the blastodisc extended radially to eventually encompass the yolk (Fig 1c) with only a small opening to the perivitelline space, the blastopore, remaining. Onset of gastrulation was apparent 7 HPF with the formation of a thickening cellular mass, the embryonic shield. Development of a discreet embryonic axis (denoting the onset of neurulation) was evident by 11 HPF (Fig 1e) and eyes were visible at 13 HPF (Fig 1f). Stellate melanophores emerged around the head at 16 HPF, and had spread along the entire body by 21 HPF. The embryo had begun to take on the appearance of a larva by 20 HPF (Fig 1g) and had begun to increase in length and deplete the yolk stores. The heart, which develops below the neural tube during gastrulation, began to beat at 25 HPF (Fig 1h) and the embryo began to move. Embryonic development was completed and hatching began to occur at 28 HPF (Fig 1i), in which 2.3 mm larvae emerged with a 0.8 mm yolk sac, non pigmented eyes and a non-patent digestive tract.

Experiment 1 - The interactive effects of ozone CT and temperature on mulloway hatching success

All groups of eggs treated with an ozone CT of 5 had a significantly poorer hatching percentage compared with eggs treated with the lower ozone CT values (Table 1). This was exacerbated at the coolest temperature (19 °C), and produced the poorest hatching percentage observed ($3.7 \pm 1.6\%$; mean \pm SEM). No significant difference in hatching percentage was found between the eggs treated with an ozone CT of 0, 0.1, 0.5 or 1 (Table 1).

Eggs treated and incubated at 25 °C hatched (50% of larvae) significantly faster than eggs maintained at 19 or 22 °C (Table 1). Eggs maintained at 22 °C and treated with a CT of 1 hatched (50% of larvae) significantly faster than eggs treated with CT values of 0.1 or 5 at 22 °C (Table 1). No difference in the hatching time (50% of larvae) was observed for any CT value at 25 °C. No significant difference in hatching time existed within the 19 °C treatments, except for those eggs treated with a CT of 5 where only 3.7 % of eggs hatched. This treatment therefore did not generate a value for the time taken for 50% of larvae to hatch.

Significant differences existed for the duration of hatching time (i.e. from the start of hatching to the end of hatching) between temperatures and CT values (Table 1). The eggs treated with a CT value of 5 typically had a significantly longer hatching duration than other CT values. No significant difference existed in hatching duration between the 0, 0.1, 0.5 or 1 CT values within the 19 °C treatment. The duration of hatching at 22 °C occurred most slowly for eggs treated with a CT value of 5. Similarly, eggs at 25 °C hatched more slowly when treated with a CT value of 5 than those treated with CT values of 0, 0.1 or 0.5. The duration of hatching time varied significantly for eggs treated with a CT value of 1 at 25 °C compared with those treated at 19 and 22°C.

3.3 Experiment 2 - The effect of ozone treatment on hatching success for mulloway eggs treated at four stages of embryonic development

No significant differences were observed between the percentage of hatched larvae when eggs were treated with ozone at any of the embryonic development stages (3, 8, 20, 27 HPF) or the CT values (CT = 0 and 1) (Table 2). No significant differences existed in the time taken for 50% of larvae to hatch for any of the embryonic development stages or the CT values (Table 2). Therefore, no significant differences in hatching success occurred for mulloway eggs treated with a CT value of 1, compared with a CT value of 0 at any of the developmental stages measured.

4. DISCUSSION

This study has determined that mulloway larvae incubated at 22°C begin to hatch from 28 HPF. Cleavage begins approximately 45 minutes post-hatching and, as is characteristic of teleost fishes in general, is meroblastic (Bone et al., 1995), and confined to the animal pole of the egg, such that cleavage is 'partial' as it does not involve the relatively large yolk mass. Successive cleavages occur every 15 min but begin to slow after the fourth division. This change in the rate of early cleavage is similar to that of yellowtail kingfish (*Seriola lalandi*), another temperate marine species, for which the rate of cleavage slows at the 32 cell stage (Moran et al., 2007). Once the blastoderm has extended to envelope the yolk, mulloway eggs develop quickly; and the embryonic shield is apparent by 7 HPF. The latter arises from the thickening of the germ ring during gastrulation, when the cells are rearranged to form three pluripotent germ layers the ectoderm, endoderm and mesoderm. These later give rise to all tissues, organs and the neural tube (Kunz, 2004; Gilbert, 2006). First, the embryonic shield elongates to form the dorsal-ventral axis of the future embryo (Kunz, 2004; Gilbert, 2006). Soon thereafter, in 'neurulation', the development of the neural tube ensues, giving rise to the central and peripheral nervous systems, associated sensory organs, and the neural chord (Gilbert, 2006); all ectodermal in origin.

Stellate melanophores appear on the head of the embryo around 16 HPF, and by 21 HPF have gradually spread along the entire body. However, pigmentation of the eyes does not occur during embryonic development but begins 1-2 days after hatching. The embryo begins to increase in length and take the form of a larval fish by 20 HPF. The increase in the length of the embryo, accompanied by depletion in the yolk volume, is common in the latter stages of development (Battle, 1944). The heart develops during gastrulation and forms in a cavity between the germ layers called the coelom (Gilbert, 2006). The heart begins to beat 25 HPF and this also coincides with the first movements of the embryo. Embryo movements later play a role in hatching, facilitating rupture of the egg envelope which has been weakened by a hatching enzyme, secreted from glands on the head of the embryo (Kunz, 2004).

The trials that followed the embryonic development study have determined that ozone can be applied to mulloway eggs and therefore may be suitable for disinfection purposes. In Experiment 1, the highest concentration of ozone used to treat mulloway eggs without negatively impacting on hatching success was a CT value of 1 (a CT value of 5 significantly reduced hatching success). This is similar to results of a study into the tolerance of gilthead sea bream (*S. aurata*) eggs to ozone, where it was determined that eggs treated with an ozone CT value greater than 1.2 had significantly reduced hatching success (Ben-Atia et al., 2007). These authors suggested the reduction in hatching may have been the result of hardening of the egg chorion by high ozone concentrations which inhibited the ability of the larvae to break through the egg surface. In our trial, the toxic effects of a high ozone CT observed for mulloway eggs was exacerbated by the lowest temperature (19 °C), where only 3.7% of larvae hatched. Ben-Atia et al. (2007) suggested that ozone tolerance is species dependent and not temperature dependent. However, for mulloway it appears that lower temperatures increased the toxic effects of ozone.

In this trial, mulloway eggs hatched more quickly and the duration of hatching was shorter when treated at 25°C than at the other temperatures. This is typical of marine teleost eggs, where hatching time is dependent on temperature (Kinne, 1963). Mulloway larvae produced at the PSFI hatchery are currently incubated and cultured at 22 °C and while hatching in this trial occurred more quickly at 25 °C, there is no apparent benefit to treating mulloway eggs at the higher temperature and therefore it is recommended that the eggs continue to be treated at the standard temperature of 22 °C.

While it is important to determine the tolerance of the eggs to ozone, it is also necessary to determine the level at which the pathogen is inactivated by ozone. Nothing is known of the effective CT value required to inactivate the Australian bass nodavirus, therefore it may be prudent to treat mulloway eggs with the maximum ozone CT value the species can tolerate in order to minimise the risk of nodavirus infection. The striped jack nervous necrosis virus (SJNNV) has been well studied and it was established that an ozone CT value of 0.25 is required to inactivate SJNNV (Arimoto et al., 1996). The inactivation of infectious pancreatic necrosis virus (IPNV) using ozone was also studied and it was found that exposure of the IPNV to ozone for 60 s at 0.1-0.2 mg/l (CT = 0.1-0.2) successfully inactivated the virus (Liltved et al., 1995). Therefore, as a CT value of 1 did not reduce hatching success, but is likely to be effective as a disinfectant, it is recommended that mulloway eggs should be disinfected using an ozone CT value of 1 in water of 22 °C. It is important to note that that this study has probably not determined the upper limit of the tolerance of mulloway eggs to ozone, which may be between an ozone CT value of 1 and 5, and further studies may determine a higher maximum tolerance level of mulloway eggs to ozone.

The ozonation protocol developed in Experiment 1 was then tested on mulloway embryos at four stages of development, 3, 8, 20 and 27 HPF, to determine if mulloway embryos were more susceptible to the effects of ozone at different stages of development. As described previously, at 3 HPF, eggs were undergoing cleavage; at 8 HPF, gastrulation had begun, the embryonic shield had developed and the neurual tube was forming (eggs in experiment 1 were also treated at this stage). At 20 HPF the embryo was in the final stages of development and had visible eyes, pigment and skeletal structures; and by 27 HPF the embryo was nearing hatching and the presence of hatching enzyme is likely (Kunz, 2004).

No differences were observed between the hatching successes of embryos treated at any of the developmental stages and as the intention of the ozone treatment is to disinfect the eggs of viruses and bacteria, it would be sensible to treat eggs early in their development. Grotmol et al. (1999) found that after inoculation with nodavirus, the incubation period of Atlantic halibut (*Hippoglossus hippoglossus*) eggs significantly reduced with increasing dose rate. It is therefore advisable to treat eggs with ozone soon after fertilisation to reduce the chance of interference to the hatching process by pathogens.

In summary, mulloway eggs incubated at 22°C begin to hatch approximately 28 HPF. Ozone disinfection up to a CT value of 1 did not reduce hatching success, while a CT of 5 produced poor hatching results. To maintain standard egg development the eggs should be treated and incubated at 22°C, which is the current protocol employed at the PSFI hatchery. Ozone toxicity (CT = 1) does not change with embryonic development and therefore it is advisable to disinfect mulloway eggs early in development to reduce adverse effects associated with viruses and bacteria on the incubation and hatching of fish eggs. The susceptibility of the Australian bass nodavirus and other pathogens to ozone still needs to be determined to ensure the protocols listed here are appropriate for disinfection. Also, the effects of ozone treatment on the post-hatching development of larvae need to be considered in future experiments.

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for combinations of temperature and ozone e 1. (Experiment 1)				
Temperature	СТ	Percent hatched	HPF to 50%	Duration of
°C		2	hatched ³	hatching $(h)^3$
19	0	97.8 ± 2.2^{a}	$33.4 \pm 0.0^{\text{y}}$	9.2 ± 1.1^{y}
	0.1	99.0 ± 0.5^{a}	$34.2 \pm 0.8^{\text{ y}}$	8.0 ± 1.1^{y}
	0.5	98.1 ± 1.9^{a}	$33.7 \pm 0.4^{\text{y}}$	6.9 ± 1.4^{xy}
	1	99.0 ± 0.6^{a}	$33.6 \pm 0.0^{\text{y}}$	$8.6 \pm 1.3^{\text{y}}$
	5	$3.7 \pm 1.6^{b y}$		
22	0	99.3 ± 0.4^{a}	$32.3\pm0.8^{ab\ y}$	$7.0\pm0.1^{ab\ y}$
	0.1	99.0 ± 1.0^{a}	$34.1 \pm 0.2^{b y}$	4.5 ± 0.8^{ax}
	0.5	100.0 ± 0.0^{a}	$32.8 \pm 1.0^{ab y}$	$7.1 \pm 0.1^{ab y}$
	1	98.9 ± 0.1^{a}	$30.6 \pm 1.0^{a x}$	$7.1 \pm 0.2^{b y}$
	5	$74.8 \pm 12.6^{b x}$	$34.6 \pm 0.5^{b y}$	$9.8 \pm 1.6^{c x}$
25	0 0.1 0.5 1 5	$\begin{array}{c} 99.2 \pm 0.4^{a} \\ 100.0 \pm 0.0^{a} \\ 99.5 \pm 0.3^{a} \\ 99.7 \pm 0.3^{a} \\ 64.6 \pm 15.3^{bx} \end{array}$	$28.7 \pm 0.2^{x} 28.9 \pm 0.1^{x} 29.5 \pm 0.1^{x} 29.1 \pm 0.3^{x} 29.8 \pm 0.7^{x} $	$\begin{array}{l} 3.6 \pm 0.7^{ax} \\ 4.0 \pm 0.4^{ax} \\ 3.6 \pm 0.9^{ax} \\ 4.4 \pm 0.1^{abx} \\ 6.5 \pm 1.1^{bx} \end{array}$

Values (mean \pm SEM; n=3) of the percentage of fish hatched, the time until 50% of larvae had hatched (hours post fertilisation) and the duration of hatching for mulloway eggs treated with ozone for combinations of temperature and ozone CT. (Experiment 1)¹

¹ Values with the same letter or no letter in the superscript within each temperature (between CT values; a, b, c) and within each CT value (between temperatures; x, y) are not significantly different (P>0.05).

² Two-factor ANOVA revealed significant effects of temperature, CT, and the interaction between temperature and CT (P<0.05). Results of one-factor ANOVA, SNK, presented.

³ One-factor ANOVA, SNK

TABLE 2

Values (mean \pm SEM; n=4) for the percentage of fish hatched, the time until 50% of larvae had hatched (hours post fertilisation) and the duration of hatching for mulloway eggs treated with ozone. (Experiment 2)¹

Treatment	HPF	Percent hatched (mean \pm SEM)	HPF to 50% hatched	Duration of hatching (h)
077 4		100 00		• • • • •
CT = 1	3	100 ± 0.0	26.1 ± 0.2	2.0 ± 0.4
	8	100 ± 0.0	27.1 ± 0.2	1.8 ± 0.3
	20	95 ± 5.0	27.9 ± 0.3	1.4 ± 0.4
	27	100 ± 0.0	27.6 ± 0.1	2.1 ± 0.6
CT = 0	3	97.5 ± 2.5	27.2 ± 0.5	1.9 ± 0.2
	8	97.5 ± 2.5	27 ± 0.3	1.9 ± 0.3
	20	85 ± 12.0	28.1 ± 0.3	1.8 ± 0.3
	27	97.5 ± 2.5	27.9 ± 0.1	1.6 ± 0.3

¹ There were no significant differences (P>0.05, two-factor ANOVA, SNK) observed between any of the values.



FIGURE 1

Embryonic development sequence of mulloway eggs incubated at 22 °C. The measurement scale is equal to 1mm. (a) Fertilised mulloway egg 0 h post fertilisation (HPF); (b) Early blastula stage was reached 4 HPF; (c) The embryonic shield appeared, indicating the onset of gastrulation, at 7 HPF; (d) Early gastrulation, in which the embryo was entering the neurula stage of development. This development stage was studied in Experiment 2; (e) The embryo had reached the neurula stage at 11 HPF, and the neural tube had appeared; (f) Muscle, skeletal structures and organs had begun to develop at 13 HPF; (g) The embryo, 20 HPF, now appearing to take the form of a larval fish, had begun to grow longer and was starting to deplete yolk stores (h) The heart began beating and the larvae began to move at 25 HPF; (i) A 2.3mm larvae hatched with a 0.8 mm yolk sac, non pigmented eyes and a disconnected digestive tract at 28 HPF.

4.2 Optimal photoperiod and light intensity for feeding and ontogeny of larval mulloway, *Argyrosomus japonicus*

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ABSTRACT

Two experiments were done to test a range of photoperiods (24L:0D; 18L:6D; 12L:12D; 6L:18D and 0L:24D) and light intensities (1 and 10 μ mol s⁻¹ m⁻²) on the growth, swim bladder (SB) inflation, food consumption and survival of early (2-8 dah [days after hatching]) and advanced (14-34 dah) mulloway larvae. The two light intensities tested had no effect on the parameters measured and there were no interactions between photoperiod and light intensity. In early larvae, length was significantly improved in fish held in 12, 18 and 24 h of light compared with fish in 6 and 0 h of light. Similarly, the number of fish that were consuming food was significantly better in 12, 18 and 24 h of light; however by 6 dah, the number of larvae consuming food had increased in the 6L:18D photoperiod. Consistently fewer larvae in the 0L:24D photoperiod consumed food throughout the trial, with the result that survival was significantly poorer in this photoperiod. Survival was also reduced in the 6L:18D photoperiod compared with the other light photoperiods. Early swim bladder inflation was significantly (P<0.05) improved in 6 h of light compared with 0, 18 and 24 h of light, but was not significantly different from fish in 12 h of light. As fish in the 12L:12D photoperiod had good growth, SB inflation and survival, it is suggested that this is appropriate until SB inflation and then the light period should be increased to 18 h of light to promote growth. In advanced larvae, no effects were observed in growth at the end of the experiment; however, survival was significantly better in the 0L:24D photoperiod compared with all of the other photoperiods. It is unknown how pigmentation and ontogeny are affected by a 0L:24D photoperiod and therefore it is recommended that an 18L:6D photoperiod is appropriate until further research is competed.

1. INTRODUCTION

Mulloway (*Argyrosomus japonicus*) have been identified as an ideal aquaculture species in Australia's southern states (Battaglene and Talbot, 1994; Fielder et al., 1999). Some attention has be given to the culture requirements of this species and previous research trials have included hormone induction and larval rearing, salinity tolerances, parasite infestations, and photoperiod and feeding requirements of juvenile fish (Battaglene and Talbot, 1994; Fielder and Bardsley, 1999; Hayward et al., 2007; Ballagh et al., 2008). Further examination of the requirements of larval mulloway is now required to increase efficiency in hatcheries by improving growth rates and reducing costs.

Some of the most widely investigated parameters for improving larval rearing protocols are photoperiod and light intensity requirements. Optimal photoperiod and light intensity can improve growth rates by increasing the feeding time available and by improving the ability of larvae to detect prey (Sayer, 1998; Boeuf and Le Bail, 1999). The primary sense mode for feeding of many species of larvae is vision, and for most species the eyes are pigmented and considered functional by first feeding (Blaxter, 1986; Pankhurst, 2008). Marine fish generally rely on a mosaic of retinal cone cells at first feeding, which allow larvae to distinguish colour and detect movement in light conditions (Campbell, 1996; Kunz, 2007; Takashima and Hibiya, 1995). Larvae later develop rod cells, which can detect the intensity of light and allow the larvae to adapt to light and dark surroundings (Blaxter, 1986; Takashima and Hibiya, 1995).

The receptivity of fish to light is different for each species and changes with ontogeny (Boeuf and Le Bail, 1999). Larvae require a minimum light intensity to properly detect and catch food, while light intensities that are too great can be stressful and cause mortality (Boeuf and Le Bail, 1999). Optimal light intensity requirements are often similar to those found in natural environmental conditions. Summer flounder (*Paralichthys dentatus*) larvae were found to perform better under low light conditions, which were consistent with the conditions of the species spawning grounds in offshore shelf waters at depths of up to 200 m (Watanabe and Feeley, 2003). The growth and survival of black sea bass (*Centropristis striata*) improved with increasing light intensities and authors reported this to be consistent with shallow, near-shore locations where the larvae are found in nature (Copeland and Watanabe, 2006).

In contrast to light intensity, improved growth and development of larvae can often be achieved by providing an artificial photoperiod. Many trials have determined that an artificially long day length stimulates growth and ontogeny in marine species (Boeuf and Le Bail, 1999). In first feeding snapper (*Pagrus auratus*) larvae, increasing photoperiod was found to promote an increase in growth and development (Fielder et al., 2002). Trotter et al., (2003) also found the growth of striped trumpeter (*Latris lineate*) larvae to be improved under a long day length (18-24 h of light) with survival greatest in 24 h of light and optimal swim bladder inflation occurring in 18 h of light.

Photoperiod is often reported to influence swim bladder (SB) inflation, which usually occurs in darkness and around the time of first feeding. An extended dark period has been observed to promote SB inflation in many marine larvae species and therefore it is necessary to consider this milestone when examining optimal photoperiod regimes for larvae (Battaglene and Talbot, 1990). A successful photoperiod regime for marine larvae is one that provides adequate darkness for SB inflation while maintaining optimal light hours for growth and survival.

The current light management protocols for intensive clear water culture of mulloway at the Port Stephens Fisheries Institute (PSFI) hatchery include: complete darkness until first feeding; a 12L:12D photoperiod from first feeding at 2 days after hatching (dah) until 10 dah; followed by an 18L:6D photoperiod. Light intensities are usually set at 1-2 μ mol s⁻¹ m⁻². Two experiments were designed to examine the light requirements of mulloway larvae. The first experiment investigated the effects of photoperiod and light intensity on the growth (length and weight), SB inflation and survival of early larval mulloway (2-8 dah), and the second experiment investigated the effects of photoperiod and light intensity on the growth (length and weight), food consumption and survival of advanced larval mulloway (14-34 dah).

2. METHODS AND MATERIALS

2.1. Fish and facilities

Fertilised mulloway eggs were sourced from a local mulloway hatchery (Clear Water Mulloway, Millers Forest, NSW). Eggs were treated with ozone (CT = 0.2) at the Port Stephens Fisheries Institute (PSFI) and were transferred to a 2000 l clear water flow-through (1.5 l/min) larviculture tank. The tank was maintained in darkness until 1 dah, at which time the water level in the tank was lowered down to condense the larvae and fish for experiment 1 were removed. The tank was then refilled and larvae were fed rotifers (*Brachionus plicitilis*) at 10/ml. The light intensity in the larviculture tank was 1 μ mol s⁻¹ m⁻². Larvae for experiment 2 were removed in a similar manner on day 13.

2.2. Experiment 1. Light intensity and photoperiod requirements of early larval mulloway

Groups of 800 larvae (1 dah); (mean length, 2.38 mm), were transferred to each of 27 100 l flowthrough (200 ml/min) experiment tanks. The tanks were kept in darkness until the following day (2 dah) when the experiment began. The experiment treatments included two light intensities (1 and 10 μ mol s⁻¹ m⁻²) and five photoperiods (24L:24D, 18L:6D, 12L:12D; 6L:18 D and 0L:24D). Black plastic sheeting surrounded each tank to exclude light leakage between adjacent tanks and daily operations within the experiment room were complete using torchlight. Lights were set to turn on immediately at 08:30, and larvae were fed nutritionally enriched rotifers (10/ml) at 0:900 and 15:00 each day. Water quality parameters were taken from 8 randomly selected tanks each day and each tank was siphoned daily to remove wastes. Larvae (20) were sampled every two days for length (mm), dry weight (mg), swim bladder (SB) inflation and the presence or absence of food. The experiment ended on day 8 (after hatching), and the total survival was assessed.

2.3. Experiment 2. Light intensity and photoperiod requirements of advanced larval mulloway

Groups of 800 larvae (13 dah) (mean length, 4.49mm) were transferred to each of 27 100 l flowthrough (2 l/min) experiment tanks. The experiment treatments included two light intensities (1 and 10 μ mol s⁻¹ m⁻²) and five photoperiods (24L:24D, 18L:6D, 12L:12D; 6L:18 D and 0L:24D). As in experiment 1, black plastic sheeting surrounded each tank to exclude light leakage and daily operations were complete using torchlight. Lights were set to turn on immediately at 08:30, and larvae were fed at 0:900 and 15:00 each day. Larvae were fed nutritionally enriched rotifers (3/ml) for the first three days of the experiment and were then maintained on nutritionally enriched *Artemia* (0.5/ml). As in experiment 1, water quality parameters were taken from 8 randomly selected tanks each day and each tank was siphoned to remove wastes. Larvae (20) were sampled every four days for length (mm), dry weight (mg) and the presence or absence of food. The experiment ended on day 34 (after hatching), and the total survival was assessed.

2.4. Water quality analyses

Water quality (means \pm SD) was measured daily using a water quality meter (Horiba U-10, Japan): pH (8.16 \pm 0.8), dissolved oxygen (8.10 \pm 0.5 mg l⁻¹), temperature (20.1 \pm 0.8 °C), and salinity (31.5 \pm 2.1‰). Water quality was consistent across all tanks for the duration of the experiment.

2.5. Statistical methods

Data were assessed for homogeneity of variance using Cochran's test (Underwood, 1997). When variances were heterogeneous, data were log transformed to establish homogeneity. The experiment was designed for 2-factor analysis of variance (ANOVA) after the exclusion of treatment 9, in which larvae were maintained in 24 h of darkness and did not include light intensity as a factor. Therefore, treatments 1-8 were analysed using 2-factor ANOVA to determine the effects of photoperiod and light intensity on length, weight, SB inflation, food consumption and survival. All treatments (1-9) were then analysed using 1-factor ANOVA to determine differences in length, weight, SB inflation, food consumption and survival between fish held in 24 h of darkness and fish held in other photoperiods. Where statistical differences were found, (P<0.05), the means were compared using the Student-Newman-Keuls (SNK) test. Statistical analyses were conducted using Statgraphics Version 4.1 (STSC, USA).

3. **RESULTS**

3.1 *Experiment 1. Light intensity and photoperiod requirements of early mulloway larvae*

Photoperiod had a significant (P < 0.05) affect on the growth, development and survival of early larvae, however there were no effects of light intensity on any of the parameters measured and there were no interactions observed between photoperiod and light intensity.

The weight (mg) of larvae increased in all treatments throughout the experiment, however no significant difference (P>0.05) was observed between the weights at any of the sample periods (Table 1). No larvae in the 0L:24D treatment were alive 8 dah, therefore no weight value is recorded.

Significant differences (P<0.05) were observed between fish lengths in different photoperiods for each sample period (Table 2). Larvae in the 6L:18D and 0L:24D photoperiods were significantly shorter than all other photoperiods for each sample period (Table 2). The samples taken at 6 and 8 dah showed significantly longer fish in the 18L:6D photoperiod than fish in the 12L:12D photoperiod. No significant difference (P>0.05) in length was observed between the 24L:0D photoperiod and the 18L:6D and 12L:12D photoperiods. No larvae in the 0L:24D treatment were alive 8 dah, therefore no length value is recorded.

Food consumption was significantly (P<0.05) affected by photoperiod. At the first sample period, 4 dah, significantly fewer larvae in the 6L:18D and 0L:24D photoperiods were consuming food than in the other photoperiods (Table 3). At the second sample, 6 dah, the number of larvae consuming food had increased in the 6L:18D photoperiod and there was no significant difference (P>0.05) between this photoperiod and the other light photoperiods (Table 3); however there were still significantly (P<0.05) fewer larvae feeding in the dark photoperiod (0L:24D) (Table 3).

Survival was significantly (P<0.05) reduced in the 6L:18D photoperiod compared with the photoperiods with 12 to 24 h of light (Table 4). Survival in the 0L:24D photoperiod was significantly poorer (P<0.05) than in all other photoperiods and no larvae remained at completion of the experiment (Table 4).

Swim bladder inflation was significantly (P<0.05) improved in the 6L:18D photoperiod compared with the 0L:24D, 18L:6D and 24L:0D photoperiods at the first sample period, 4 dah (Table 4). No significant difference (P>0.05) existed in swim bladder inflation between fish in the 12L:12D photoperiod and the other photoperiods (Table 4). SB inflation was completed in all photoperiods by 6 dah.

3.2 Experiment 2. Light intensity and photoperiod requirements of advanced mulloway larvae

As in experiment 1, photoperiod had a significant (P<0.05) affect on the performance of advanced larvae, however there were no affects of light intensity on any of the parameters measured and there were no interactions observed between photoperiod and light intensity.

The weights of larvae were not significantly different (P>0.05) at completion of the experiment. The only difference observed in fish weight occurred at 22 dah, at which time the larvae in the 18L:6D photoperiod were significantly heavier than those in the 6L:18D and 0L:24D photoperiods (Table 5).

Larvae lengths were also not significantly different (P>0.05) at the end of the experiment, however significant differences (P<0.05) did exist for the first three sample periods (Table 6). Larvae in the long light photoperiods (18L:6D and 24L:0D) were significantly (P<0.05) longer than those in the long dark photoperiods (6L:18D and 0L:24D) at 18, 22, 26 dah (Table 6).

The percentage of larvae consuming food was high throughout the experiment and was not affected by photoperiod or light intensity. The percentages (mean \pm SEM) of fish feeding ranged from 90.0 \pm 7.6 to 100 \pm 0.0 % (Table 7). All fish were observed to be feeding from 26 dah. Survival was significantly improved in the 0L:24D photoperiod compared with survival in all other photoperiods (Table 7).

4. DISCUSSION

The first part of this study has determined that length, survival, food consumption and swim bladder inflation of early larval mulloway were influenced by photoperiod; however there were no observed effects of the light intensities tested. Larvae in the 6L:18D and 0L:24D photoperiods were significantly shorter than fish held in longer light periods, which is similar to many studies that have reported poor growth in photoperiods with long dark periods and attributed this to the lack of

feeding time available to larvae that rely on vision for feeding (Cobcroft and Pankhurst, 2003; Yoseda et al., 2008). No significant difference was observed between weights of larvae; however as the mean dry weights of larvae were as little as 0.022 mg, it is likely that unavoidable error in the weighing process may have influenced these results.

Food consumption was low in larvae maintained in the 0L:24D and 6L:18D photoperiods at the first sample period (4 dah), however a small percentage of larvae did consume food. The incidence of food consumption in larvae fed during darkness has been identified before and Huse (1994) suggested the cause may be accidental ingestion through osmoregulatory drinking. The percentage of larvae consuming food in the 6L:18D photoperiod increased considerably by the second sample period (6 dah), and therefore significantly more larvae were consuming food in the light photoperiods than in the 0L:24D photoperiod. The continued lack of food ingested by larvae in the 0L:24D photoperiod resulted in poor survival and by the third sample period (8 dah), no larvae had survived. Similar results have been reported before with many marine larval species suffering mortality through inadequate food consumption in dark conditions. A study into Atlantic cod (Gadus morhua) found reduced survival in short light photoperiods and authors attributed this to a shorter feeding period and less chance of encountering prey (Puvanendran and Brown, 2002). Similarly, food consumption and survival in vundu catfish (Heterobranchus longifilis) larvae was reduced in long dark periods compared with long light periods and authors suggested this was the result of restricted visibility (Nwosu and Holzlöhner, 2000). The reduced growth and survival in mulloway larvae held in long dark photoperiods provides evidence that early larval mulloway are predominantly visual feeders.

SB inflation was also influenced by photoperiod, and at the first sample period (4 dah), a significantly greater percentage of larvae had inflated their SB in the 6L:18D photoperiod compared with larvae in the 0L:24D, 18L:6D and 24L:0D photoperiods, while SB inflation of larvae held in the 12L:12D photoperiod was not significantly different from fish in any other photoperiod. Improved SB inflation in photoperiods with long dark periods has been observed in Australian marine larval species before. Trotter et al., (2003) observed behavior associated with SB inflation during the dark-phase in larval striped trumpeter (*Latris lineate*), while Battaglene et al., (1994) also observed SB inflation after the onset of darkness in sand whiting (*Sillago ciliata*). Inflation of the SB after the onset of darkness may be an adaptation for predator avoidance, as was suggested by Martin-Robichaud and Peterson (1998). SB inflation in mulloway larvae was discussed by Battaglene and Talbot (1994), who observed that poor SB inflation resulted in reduced growth and survival. It is therefore suggested that SB inflation should be promoted by providing an adequate dark phase to ensure growth and survival are not adversely affected.

The optimal photoperiod for early mulloway larvae would include a long dark phase to ensure SB inflation, in conjunction with an adequate light phase to enhance feeding time and improve survival. As fish in the 12L:12D photoperiod did not perform poorly in any of the parameters measured but had adequate darkness for swim bladder inflation and enough light to allow for feeding it is the most suitable photoperiod for early larvae. However, as growth was improved in the long light photoperiods it is suggested that the photoperiod be adjusted to 18L:6D once SB inflation has been completed.

The effects of photoperiod and light intensity on advanced mulloway larvae were different to those observed for the early larvae trial. Growth (weight and length) in the long dark photoperiods (0L:24D and 6L:18D) was not significantly different to those of the long light photoperiods at the completion of the experiment, however differences were observed in early samples. As larvae developed and increased in length, their apparent ability to perform well in long dark periods improved. Larvae held in the longer dark periods had significantly poorer growth up until, and including, the third sample period (26 dah); however, by the fourth sample period (30 dah) no differences existed between the lengths or weights of larvae in any of the photoperiods. Marine fish
predominantly rely on vision for prey detection; however, mechanoreception and chemoeception play an increasingly important role in feeding as larval development proceeds (Blaxter, 1986; Pankhurst, 2008). As the suite of sensory organs develops, the feeding opportunities available to larvae increase (Cobcroft and Pankhurst, 2003). The improved function of the sensory organs may have increased the feeding ability of larvae in the dark phases, and may explain the improved growth rates in fish from the 0L:24D and 6L:18D photoperiods.

In addition to improved growth rates, the survival of larvae maintained in the 0L:24D photoperiod was significantly better than all other photoperiods. The improved feeding ability of larvae is likely to have contributed to this increase in survival percentage but it does not explain why survival was better than the other photoperiods. Cannibalism was reported by Battaglene and Talbot (1994) to be a significant contributor to mortality of larval mulloway. Mulloway have been observed in larval rearing tanks to cannibalise larvae tail first in contrast to many marine fish which cannibalise head first. It is thought that the shape of larval mulloway makes it difficult for them to be consumed head first. Cannibalism may be restricted in darkness as vision is likely to play an important role in cannibalistic capture and consumption and therefore may have contributed to the increased survival.

In addition to the role light plays in feeding and survival of larval fish, pigmentation and digestive enzyme activity can also be affected by light (Boeuf and Le Bail, 1999; Shan et al., 2008). It is unclear what affect 24 h of darkness will have on the pigmentation and enzymatic activity of mulloway and therefore it is suggested that the photoperiod should include some light.

In summary, early larval mulloway require an adequate dark phase for successful SB inflation but growth and survival are improved in longer light photoperiods. Therefore, as larvae in the 12L:12D photoperiod had successful SB inflation, good growth rates and good survival, it is suggested that larvae are maintained in a 12L:12D photoperiod from first feeding until SB inflation is complete. The light regime should then be changed to a 18L:6D photoperiod to maximize feeding time and growth rates and also to increase survival rates. In the second experiment, it was determined that advanced mulloway larvae are able to feed in light and dark conditions. As it was determined that 18 h of light was the most suitable photoperiod for growth in early larvae and it has been reported that 18 h of light is also appropriate for rearing juvenile mulloway (Ballagh et al., 2008), it is suggested that advanced larvae should also be reared in 18 h of light. Further investigations into the light intensity tolerances need to be completed to gain a better understanding of larval feeding behaviour.

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Values are weights (mg) (means \pm SEM) for each photoperiod and light intensity at each sample period (4, 6 and 8 days after hatching [dah]) in experiment 1. No significant difference was observed between the larval weights at any of the sample periods; no significant difference was observed between light intensities.

Photoperiod (h)	Light intensity (µmol)	4 dah	6 dah	8 dah
24L:0D	1	0.035 ± 0.005	0.058 ± 0.008	0.068 ± 0.012
	10	0.044 ± 0.004	0.061 ± 0.001	0.074 ± 0.007
18L:6D	1	0.040 ± 0.001	0.043 ± 0.002	0.080 ± 0.012
	10	0.027 ± 0.014	0.049 ± 0.008	0.069 ± 0.007
12:L12D	1	0.035 ± 0.012	0.047 ± 0.011	0.061 ± 0.006
	10	0.035 ± 0.003	0.048 ± 0.001	0.056 ± 0.004
6L:18D	1	0.030 ± 0.003	0.047 ± 0.006	0.064 ± 0.009
	10	0.022 ± 0.003	0.043 ± 0.007	0.056 ± 0.000
0L:24D	0	0.029 ± 0.009	0.039 ± 0.005	-

TABLE 2

Values are lengths (mm) (means \pm SEM) for each photoperiod and light intensity at each sample period (4, 6 and 8 days after hatching [dah]) in experiment 1. Photoperiods with the same letter in the superscript are not significantly different; no significant difference was observed between light intensities.

Photoperiod (h)	Light intensity (µmol)	4 dah	6 dah	8 dah
24L:0D	1 10	2.8 ± 0.0^{b} 2.8 ± 0.0^{b}	3.2 ± 0.0^{b} 3.3 ± 0.0^{b}	3.6 ± 0.1^{bc} 3.7 ± 0.0^{bc}
18L:6D	1 10	$\begin{array}{l} 2.8\pm0.0^b\\ 2.8\pm0.0^b\end{array}$	3.4 ± 0.1^{c} 3.4 ± 0.1^{c}	$3.8 \pm 0.1^{\circ}$ $3.7 \pm 0.0^{\circ}$
12L:12D	1 10	2.8 ± 0.1^{b} 2.8 ± 0.0^{b}	3.2 ± 0.1^{b} 3.2 ± 0.0^{b}	3.6 ± 0.0^{b} 3.6 ± 0.1^{b}
6L:18D	1 10	2.6 ± 0.0^{a} 2.6 ± 0.1^{a}	$\begin{array}{l} 2.6\pm0.1^a\\ 2.8\pm0.0^a\end{array}$	3.0 ± 0.0^{a} 3.0 ± 0.0^{a}
0D:24D	0	2.6 ± 0.1^{a}	$2.8\pm0.0^{\text{a}}$	-

Values are the incidence of food consumption (means \pm SEM) as a percentage of fish for each photoperiod and light intensity. Photoperiods with the same letter in the superscript are not significantly different; no significant difference was observed between light intensities.

Photoperiod (h)	Light intensity (µmol)	4 dah	6 dah	8 dah
24L:0D	1	94.0 ± 6.0^{b}	$78.0 \pm 1.3^{\text{b}}$	100.0 ± 0.0
	10	$88.0 \pm 11.7^{\mathrm{b}}$	$98.3\pm0.2^{\rm b}$	100.0 ± 0.0
18L:6D	1	$86.7\pm10.0^{\rm b}$	96.0 ± 0.4^{b}	96.7 ± 0.3
	10	97.7 ± 2.3^{b}	100.0 ± 0.0^{b}	100.0 ± 0.0
12:L12D	1	90.3 ± 2.3^{b}	75.0 ± 1.0^{b}	93.3 ± 0.1
	10	95.7 ± 2.2^{b}	89.3 ± 1.1^{b}	92.7 ± 0.4
6L:18D	1	2.3 ± 2.3^a	69.0 ± 1.3^{b}	61.7 ± 1.2
	10	3.0 ± 2.4^{a}	71.0 ± 0.4^{b}	81.7 ± 0.3
0L:24D	0	6.0 ± 3.1^{a}	12.0 ± 0.3^a	-

TABLE 4

Values are the survival percentage (means \pm SEM) for each photoperiod and light intensity at completion of the experiment; and the percentage of fish that had completed SB inflation (mean \pm SEM) at 4 dah (experiment 1). Photoperiods with the same letter in the superscript are not significantly different; no significant difference was observed between light intensities.

Photoperiod (h)	Light intensity (µmol)	% Survival	% SB Inflation
24L:0D	1	$15.4 \pm 3.5^{\circ}$	82.0 ± 3.2^{a}
	10	$20.0 \pm 11.5^{\circ}$	89.0 ± 0.6^{a}
18L:6D	1	$29.2 \pm 6.1^{\circ}$	78.3 ± 5.8^{a}
	10	$33.8 \pm 2.6^{\circ}$	93.3 ± 4.1^{a}
12:L12D	1	$20.5\pm5.3^{\text{c}}$	94.0 ± 3.5^{ab}
	10	$16.1 \pm 3.3^{\circ}$	89.3 ± 3.6^{ab}
6L:18D	1	4.9 ± 1.6^{b}	98.0 ± 2.0^{b}
	10	$6.8\pm1.3^{\rm b}$	$100\pm0.0^{\mathrm{b}}$
0L:24D	0	0.0 ± 0.0^{a}	$88.3\pm3.3^{\text{a}}$

Values are weights (mg) (means \pm SEM) for each photoperiod and light intensity at each sample period (18, 22, 26, 30 and 34 dah [dah = days after hatching]) in experiment 2. Photoperiods with the same letter in the superscript are not significantly different; no significant difference was observed between light intensities.

Photoperiod (h)	Light intensity (µmol)	18 dah	22 dah	26 dah	30 dah	34 dah
24L:0D	1	0.58±0.30	0.70±0.05 ^{ab}	1.60±0.15	3.22±0.15	5.25±0.00
	10	0.51±0.03	$0.75{\pm}0.02^{ab}$	1.55±0.29	3.44±0.50	6.60±0.69
18L:6D	1	0.50±0.15	$0.68{\pm}0.02^{b}$	1.31±0.05	2.28±0.23	4.54±0.08
	10	0.43±0.06	$0.77{\pm}0.04^{b}$	1.62±0.35	3.26±0.77	5.46±1.00
12L:12D	1	0.36±0.00	$0.63{\pm}0.06^{ab}$	1.31±0.02	2.43±0.11	4.97±0.47
	10	0.45±0.10	$0.54{\pm}0.05^{ab}$	1.47 ± 0.10	2.34 ± 0.04	3.87±0.00
6L:18D	1	0.26±0.00	$0.53{\pm}0.01^{a}$	1.2±0.05	2.76±0.12	4.84±0.24
	10	0.24±0.03	$0.49{\pm}0.02^{a}$	1.01 ± 0.06	1.72 ± 0.03	3.47 ± 0.00
0L:24D	0	0.54±0.23	0.47±0.02 ^a	1.02±0.14	1.87±0.12	3.51±0.55

TABLE 6

Values are lengths (mm) (means \pm SEM) for each photoperiod and light intensity at each sample period (18, 22, 26, 30 and 34 dah [dah = days after hatching]) in experiment 2. Photoperiods with the same letter in the superscript are not significantly different; no significant difference was observed between light intensities.

Photoperiod (h)	Light intensity (µmol)	18 dah	22 dah	26 dah	30 dah	34 dah
24L:0D	1	5.7 ± 0.2^{b}	7.7 ± 0.2^{bc}	9.9 ± 0.4^{b}	12.2 ± 0.3	14.2 ± 0.0
	10	5.7 ± 0.0^{b}	7.6 ± 0.3^{bc}	9.6 ± 0.2^{b}	12.3 ± 0.3	15.1 ± 0.4
18L:6D	1	5.6 ± 0.1^{b}	$7.7 \pm 0.1^{\circ}$	9.1 ± 0.1^{ab}	11.4 ± 0.1	13.8 ± 0.2
	10	$5.5\pm0.1^{\text{b}}$	$7.8 \pm 0.1^{\circ}$	9.7 ± 0.4^{ab}	12.2 ± 0.9	14.5 ± 0.5
12L:12D	1	5.2 ± 0.1^{a}	7.4 ± 0.2^{b}	9.1 ± 0.1^{ab}	11.4 ± 0.0	14.5 ± 0.2
	10	5.1 ± 0.0^{a}	7.1 ± 0.2^{b}	9.2 ± 0.1^{ab}	11.4 ± 0.0	13.3 ± 0.0
6L:18D	1	5.3 ± 0.1^{a}	6.8 ± 0.1^{a}	8.9 ± 0.1^{a}	11.3 ± 0.3	14.1 ± 0.4
	10	5.2 ± 0.1^{a}	6.7 ± 0.2^{a}	8.4 ± 0.2^{a}	10.6 ± 0.3	11.6 ± 1.0
0L:24D	0	$5.0\pm0.1^{\text{a}}$	6.5 ± 0.1^{a}	$8.2\pm0.4^{\text{a}}$	10.5 ± 0.4	12.8 ± 0.4

Values are the survival percentage (means \pm SEM) for each photoperiod and light intensity in experiment 2. Photoperiods with the same letter in the superscript are not significantly different; no significant difference was observed between light intensities.

Photoperiod (h)	Light intensity (µmol)	% Survival
24L:0D	1 10	$\begin{array}{c} 0.7\pm0.7^a\\ 9.7\pm3.0^a\end{array}$
18L:6D	1 10	18.6 ± 4.6^{a} 23.3 ± 9.9^{a}
12L:12D	1 10	14.3 ± 9.7^{a} 1.5 ± 1.5^{a}
6L:18D	1 10	21.3 ± 2.5^{a} 2.8 ± 2.6^{a}
0L:24D	0	54.5 ± 12.9^{b}

¹Data were log transformed to homogenize variances.

4.3 Weaning requirements of larval mulloway, Agyrosomus japonicus

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ABSTRACT

Mulloway (Argyrosomus japonicus) is an emerging aquaculture species in Australia, but there is a need to improve production technology and lower costs, including those associated with live feeds and larval rearing. Three experiments were done to determine appropriate weaning strategies from live feeds, rotifers (Brachionus plicatilis) and Artemia, to cheaper formulated pellet diets. Experiment 1 examined the effects of feeding Artemia at different levels (0, 50 or 100% ration of Artemia fed from 18 dah; based on industry protocol) and a standard quantity of pellet diet from two larval ages (14 or 23 dah). In addition, rotifers were fed to all treatments for the duration of the experiment; 29 dah, at which time all larvae were successfully weaned onto a pellet diet. Growth of larvae was significantly (P < 0.05) greater in treatments fed Artemia a 50% or 100% ration than those in the 0% ration. Experiments 2 and 3 were designed to determine the size (total length [TL], mm) at which mulloway larvae selected Artemia in preference to rotifers, and pellet (400µm) in preference to Artemia, respectively. Each day larvae were provided with either rotifers (2/ml), Artemia (2/ml) or a combination of rotifers (1/ml) and Artemia (1/ml) for 10 days (Experiment 2); and Artemia (2/ml), pellet diet or a combination of Artemia (1/ml) and pellet diet which was broadcast to excess every 15 min for 15 days (Experiment 3). After 1 h, larvae (n=20) were randomly selected and the gut contents observed under a light microscope. Mulloway larvae began consuming Artemia in combination with rotifers at 4.4±0.4 mm TL, and by 5.2±0.5 mm TL were selecting Artemia in preference to rotifers. Mulloway larvae began consuming pellet in combination with Artemia at 7.6±1.0 mm TL and by 10.6±1.8 mm TL were selecting pellet in preference to Artemia.

1. INTRODUCTION

Mulloway (*Argyrosomus japonicus*) naturally occurs in Australia's southern coastal waters and have aquaculture potential in NSW (Fielder et al., 1999). Aquaculture, of this and other finfish species in Australia is limited by high production costs. Some of the most significant costs are attributed to feeding, particularly those associated with live feeds, such as rotifers and *Artemia*, required to rear early larvae.

It is well documented that the cost and quality of *Artemia* can fluctuate over time as the supply is dependent on both worldwide aquaculture demand and weather patterns affecting the primary harvest areas, in particular the Great Salt Lake which provides 85-90% of the world's *Artemia* (Sorgeloos et al., 2001; Callan et al., 2003). In addition to the unstable nature of *Artemia* supply, the percentage of hatchery feed costs attributed to *Artemia* are substantial. Person Le Ruyet et al., (1993) reported that although live feeds contribute to only 1.6% of the total amount of dry food required to rear three month old juvenile sea bass (*Dicentrarchus labrax*), it made up to 50% of the total feeding costs. As *Artemia* contribute to such a large proportion of the larval feeding costs and the supply is unreliable, it is desirable to find alternative feeding strategies for rearing marine fish.

While there is a strong argument to reduce reliance on *Artemia*, some risks are associated with early weaning and reduced *Artemia* use. Person Le Ruyet et al., (1993) reported that *Artemia* savings could be as high as 80% by weaning sea bass 15 days earlier than the usual protocol; however the risks introduced include a decrease in weight gain, increased size variation and

skeletal abnormalities. Current larval weaning research endeavours to reduce feed costs while overcoming these problems associated with a decrease in the use of *Artemia* (Callan et al., 2003; Curnow et al., 2006; Fletcher et al., 2007).

Weaning success of any finfish species onto a formulated microdiet is partly dependent on the composition of the diet and the ability of the larvae to select and digest a non-live food (Person Le Ruyet et al., 1993; Kolkovski, 2001; Shaw et al., 2003). Stomach development and the availability of digestive enzymes are regarded as indicators for the transition from live feeds to microdiets to begin (Watanabe & Kiron, 1994; Cahu & Infante, 2001; Chen et al., 2006); however the rate of ontogeny, including development of digestive enzyme secretion, varies between species (Kolkovski, 2001). It is believed that larvae are able to digest live feeds more effectively than microdiets because they have insufficient digestive enzymes to break down a pellet which contains 60-90% dry matter in comparison with 10% dry matter in zooplankton (Kolkovski, 2001), and additionally microdiets contain ingredients such as binders and proteins which are difficult to digest (Lindner et al., 1995; Partridge and Southgate, 1999). It has also been suggested that live feeds support larval digestion by donating their own enzymes to aid the digestive process (Kolkovski, 2001), and by stimulating enzyme secretion from the pancreas and activating zymogens in the gut to increase overall enzymatic activity (Person Le Ruyet et al., 1993).

Food selection and acceptance play an important role in maximising growth rates and weaning success. Selective feeding has been described as an evolutionary tendency to maximise energy intake and must be an adaptive feature of larval fish to optimise energy consumption (Greene, 1986; Schoener, 1987). Shifts in food type preference have been measured against larval age (Hung et al., 2002, Shaw et al., 2003), weight (Olsen et al., 2000), and length (Mayer & Wahl, 1997), but the underlying factor that links these parameters & and Janssen, 1992; Pankhurst, 2008), locomotive ability and mouth gape all play a role in prey detection, capture and consumption, but in addition the prey taxa, size and abundance can influence preference selection (Mayer & Wahl, 1997; Pryor & Epifanio, 1993). Olsen et al. (2000) suggested that prey colour and swimming speed also influenced prey selection by Atlantic halibut (*Hippoglossus hippoglossus*), which can also be related to ontogeny and the ability of larvae to identify and capture prey.

Weaning success and feeding efficiency can be improved by introducing new food sources at appropriate stages of larval development. At the Port Stephens Fisheries Institute (PSFI) mulloway hatchery approximately 500,000 larvae are typically stocked into either a 2,000 l intensive clear water tank or a 10,000 l green water tank (with resident algae). The density of live prey is increased with larval density. Current weaning protocols use large strain rotifers (*Brachionus plicatilis*) from first feeding (2 days after hatching [dah], 2.5mm in length[TL]) at a rate of 4/ml for a 10,000 l tank and up to 20/ml for a 2000 l tank. When larvae attain a mean length of 4-5 mm, rotifers are supplemented with nutritionally enriched *Artemia*, which are initially introduced at a low rate (0.2-1/ml) to reduce the chance of residual non-enriched *Artemia* being consumed by larvae. The *Artemia* density is then increased to accommodate the increased demand as larvae develop. A 400 µm weaning diet is introduced when fish attain lengths of approximately 8 mm, and the concentration of *Artemia* is gradually reduced until larvae are considered to be weaned (when all sampled fish are consuming mostly pellet).

Three experiments were conducted to examine if mulloway weaning practices can be improved to reduce costs by reducing the amount of *Artemia* required during the weaning process. The first experiment aimed to investigate the effects of a range of live feed and pellet combinations on growth and weaning success of larval mulloway. The second and third experiments aimed to investigate the transition by larval mulloway from rotifers to *Artemia* and then from *Artemia* to a pellet microdiet. The outcomes of the *Artemia* and pellet transition trial were then validated in a pilot scale production run.

2. METHODS

2.1 Fish and facilities

Captive mulloway broodstock held at the Port Stephens Fisheries Institute (PSFI), NSW, Australia were induced to spawn using temperature cues. Fertilised eggs were collected on four occasions, quantified and then treated with ozone (CT=0.2), before being transferred to a 2000 l flow-through clear water (1.5 l/min) larval rearing tank for both Experiments 1 and 2, and an 8000 l flow through (5 l/min) larval rearing tank for Experiment 3 and the validation trial.

2.2 Experiment 1

Experiment 1 examined the effects of feeding six different combinations of live feeds and a pelleted microdiet (3/5 Proton, INVE, Dendermonde, Belgium) on the weaning success and growth of larval mulloway. Larvae were maintained on rotifers at 10/ml until 13 dah, when 800 larvae were stocked into each of 30 x 100 l flow through (200 ml min⁻¹) experiment tanks (n=5 replicate tanks/treatment). The experiment treatments (Table 1) began the following day and continued until larvae in all treatments were considered to be successfully weaned. Each experiment tank was siphoned daily to remove excess food and waste and the internal overflow screens were cleaned. Lights were set to turn on and off immediately without a dimming effect at 08:00 h and 20:00 h respectively (Australian Eastern Standard Time; 8 µmol m⁻¹ s⁻¹ in the light phase). Rotifers were fed to all treatments twice daily (0900 h and 1500 h) throughout the experiment (Table 1). Nutritionally enriched Artemia were fed twice daily to four of the six treatments from 18-27 dah at either a standard industry ration or half the industry ration. The standard industry ration began at 0.4/ml per feed at 18 dah (5 \pm 0.13 mm, mean larvae length) and was doubled each day until 21 dah. The ration was then reduced by half each day until 27 dah, which was the last feed of Artemia. The pelleted microdiet was broadcast four times daily from either 14 or 23 dah until completion of the experiment. The quantity of pellet increased with biomass in each tank (approximately 17%) each day. Larvae (n = 20) were sampled every four days and euthanased using a lethal dose of ethyl-p-aminobenzoate (100 mg/l). Fish were immediately assessed using a dissection microscope (M5 - 89734, Wild Heerbrugg, Switzerland) for total length (mm) from the top of the snout to the end of the tail, and food type consumed. The 20 fish from each tank were then pooled and dried (16 h, 105 °C) to obtain the mean dry weight (g) of fish. Survival was calculated for each treatment replicate at the end of the experiment and excluded the fish removed for sampling. Percent survival = (Final no. of fish / 920) * 100.

2.3 Experiment 2

Experiment 2 examined the transition of mulloway larvae from rotifers to enriched Artemia. Larvae for the experiment were sourced from a 2000 l flow-through clear water larviculture tank where they were maintained on rotifers at a density of 10/ml. Prior to and during the experiment, larvae in the holding tank were given one feed of Artemia each day at a density of 0.1/ml so they were not naive to the new food source at commencement of the experiment protocols. The tank water was exchanged at a rate of 1.5 l/min, which ensured clearance of live feeds from the tank during the night. Twenty fish were sampled daily and once the mean length reached 4.1mm, on each morning thereafter, and prior to the addition of food in the tank, a sub-sample of larvae (n=20) was obtained to confirm that no residual food remained in the digestive tract from the previous day. Then, 100 larvae were transferred everyday to each of 15 x 10 l lightly aerated, randomly positioned blue experiment vessels (ambient light conditions, similar to that of the larviculture tank). Larvae were acclimated for 30 minutes before they were fed either rotifers at 2/ml (treatment 1), Artemia at 2/ml (treatment 2) or both rotifers and Artemia at 1/ml each (treatment 3). Larvae were given 1 h to feed and were then euthanased using a lethal dose of ethyl-p-aminobenzoate (100 mg/l) and immediately preserved in 10% buffered formalin for later dissection and stomach content assessment. Twenty fish from each replicate were examined using a dissection microscope (M5 – 89734, Wild Heerbrugg, Switzerland) and the food type consumed by individual larvae was recorded.

2.4 Experiment 3

Experiment 3 examined the transition of mulloway larvae from enriched Artemia to a pellet diet. Larvae for the experiment were sourced from an 8000 l flow-through holding tank, where they were maintained on Artemia at a density of 1/ml. Leading up to and during the experiment, larvae in the holding tank were broadcast fed a 300-500 µm pellet (3/5 Proton, INVE, Dendermonde, Belgium) once each day so they were not naive to the new food source. Once the mean total length of mulloway reached 8.3 ± 0.3 mm, each day thereafter, and prior to addition of food in the larvae tank, 100 larvae were transferred to each of 15 x 10 l lightly aerated, randomly positioned experiment vessels (ambient light conditions, similar to that of the holding tank). Clearance of food from the guts of larvae was confirmed prior to transfer, as described in experiment 2. Larvae were acclimated for 30 min before being offered either Artemia at 2/ml (treatment 1), a 400 µm pellet broadcast in 15 min intervals (Treatment 2) or both Artemia at 1/ml and pellet broadcast in 15 min intervals (Treatment 3). The amount of pellet provided was equivalent to the number of Artemia provided. Larvae were allowed to feed for 1 h and were then euthanased using a lethal dose of ethyl-p-aminobenzoate and were immediately preserved in 10% formalin for later dissection and stomach content assessment. As in Experiment 2, 20 fish from each replicate were examined using a dissection microscope and food type consumed by individual larvae was recorded.

Experiment 3 showed that the transition from *Artemia* to pellets occurred once the size of mulloway was 10.6mm. These optimal feeding outcomes were then confirmed in a production run at the PSFI. Mulloway larvae were housed in 2 x 8000 l tanks and maintained on enriched *Artemia* at 1/ml. Once the average length of fish within the tanks was 9.8 ± 1.1 mm (mean \pm SD) the larvae were offered a 300-500 µm pellet diet (3/5 Proton, INVE, Dendermonde, Belgium) in addition to the 1/ml ration of *Artemia*. The ration of *Artemia* was reduced over the following three days to 0.8/ml, 0.6/ml and 0.3/ml respectively. The larvae were then maintained on *Artemia* at 0.3/ml until all fish were observed to be feeding on pellets (seven days from the initial addition of pellet). Pellets were distributed to each tank using an automatic belt feeder and through regular manual broadcasting. Twenty larvae were sampled daily from each tank and the number of pellet and *Artemia* in individual larvae and total length was recorded. The total length was recorded for every fish, which differs from the initial feed selection experiments where the mean length of fish in the holding tank was recorded each day.

2.5 Water Quality Analysis

Water quality (mean \pm SD) was measured daily in Experiment 1 using a water quality meter (Horiba U-10, Japan): pH (8.18 \pm 0.07), dissolved oxygen (6.69 \pm 0.33mg l⁻¹), temperature (26.3 \pm 0.77°C), and salinity (35.3 \pm 0.12‰). Total ammonium (NH₄⁺) (0.1 \pm 0.0mg l⁻¹) was measured daily in 4 randomly selected tanks using a rapid test kit (E.Merck, Model 1.08024, Germany). Water quality parameters were consistent across all treatment tanks for the duration of the experiments. Water quality in Experiment 2 were: pH (8.1 \pm 0.1), dissolved oxygen (7.5 \pm 1.6 mg l⁻¹), temperature (20.1 \pm 1.4°C), salinity (32 \pm 0.4‰), total ammonium (0.06 \pm 0.08 mg l⁻¹). Experiment 3 water quality parameters were: pH (8.1 \pm 0.2), dissolved oxygen (7.6 \pm 0.2 mg l⁻¹), temperature (22.5 \pm 0.8°C), salinity (30.4 \pm 0.8‰), total ammonium (0.40 \pm 0.15 mg l⁻¹). The validation experiment water quality parameters were: pH (7.8 \pm 0.1), dissolved oxygen (10.1 \pm 1.0 mg l⁻¹), temperature (21.3 \pm 0.3°C), salinity (35.7 \pm 0.1‰).

2.6 Statistical analyses

Statistical analyses were conducted using Statgraphics Version 4.1 (STSC, USA). In Experiment 1, data were analysed for homogeneity of variance using Cochran's test. The experiment was designed for two-factor analysis of variance (ANOVA) to determine the effects of each treatment on weight, length and survival. Where statistical differences were found (P<0.05), the means were compared using the Student-Newman-Keuls test (SNK).

In Experiments 2 and 3, data in treatment 3 were analysed for the mean number of food items consumed. Data were compared using a two sample paired t-test. Chesson's selectivity index (α) (Chesson, 1978) was also used to compare feeding preferences of mulloway larvae for each prey type.

$$\alpha_i = \frac{r_i / n_i}{\sum_{i=1}^m r_i / n_i}, \quad i = 1, \dots m$$

Where r_i is the number of items of prey type *i* in the larvae diet, n_i is the number of items of prey type *i* in the environment and *m* is the total number of prey types. Only larvae that had selected one or more prey items were used in the calculation of the selection index, therefore non-feeding fish were excluded from the analysis. Feeding preferences were then compared to a neutral selection ($\alpha = 0.5$) using a t-test.

The percentage of fish feeding on each prey type was recorded for all treatments in Experiments 2 and 3 to determine feeding ability of larval mulloway.

The percentage of fish feeding on each prey type in Treatment 3 (offered two food types) were compared using a paired t-test, all other comparisons were completed using a t-test.

3. **RESULTS**

3.1 Experiment 1.

No interactions existed between the two factors, pellet (early or late) and *Artemia* (0, 50 or 100% ration) for weight or length. Also, no effects of the two levels of pellet were observed for weight or length. Fish that were not fed *Artemia* performed poorly when compared to treatments that were fed *Artemia*. Significant differences (P<0.05) existed between the mean weight and length of fish in treatments that were not fed *Artemia*, (i.e. Treatments 3 and 6) and the remaining four treatments (Figure 1; Figure 2; Table 2). However, no significant differences (P>0.05) in mean weight or length were found between treatments fed a half ration (Treatments 2 and 5) or a full ration (Treatments 1 and 4) of *Artemia* (Figure 1; Figure 2; Table 2).

Fish in treatments that were not fed *Artemia* began consuming pellet before fish that were fed *Artemia*. At 21 dah, two replicates from each of treatments 5 and 6 contained fish that were feeding on minimal amounts of pellet. The average length (mean \pm SEM) of fish in these treatments was 7.7 \pm 0.4 mm and 7.2 \pm 0.1 mm respectively (Figure 3). At 25 dah, mean lengths of fish in treatments 3 and 6 (not fed *Artemia*) were 8.8 \pm 0.3 mm and 9.6 \pm 0.3 mm respectively, and all replicates contained fish that were feeding on pellets, however no fish in Treatment 5 had consumed pellets in this sample period. All fish in all treatments were feeding on pellet by 29dah, where mean lengths ranged from 10 ± 0.3 mm (treatment 3) to 14.5 ± 0.5 mm (Treatment 4).

An interaction occurred for survival between the two factors *Artemia* (0, 50 or 100% ration) and pellets (14 or 23 dah) (P = 0.035). Fish fed pellets from 23 dah had poorer survival than fish fed pellets from 14 dah when they were also fed a 0 or 50% ration of *Artemia*. However, those fish fed 100% of the *Artemia* ration had better survival when offered pellets from 23 dah compared with fish fed pellets from 14 dah. Although this interaction occurred, there were no significant effects of Artemia, only of pellets (P<0.05).

3.2 Experiment 2. Mulloway larvae preference selection of rotifers and Artemia

The (mean \pm SEM) percentage of larvae feeding in Treatment 1 (offered only rotifers) ranged from 21 \pm 0.9 to 88 \pm 1.0% in fish with mean lengths of 4.1 \pm 0.3 and 5.4 \pm 0.5 mm, respectively (Table 3). The percentage of larvae feeding in Treatment 2 (offered only *Artemia*) increased throughout the experiment from 11 \pm 0.7 to 81 \pm 0.9 % in fish with mean lengths of 4.1 \pm 0.3 and 5.5 \pm 0.4 mm, respectively (Table 3). Mulloway larvae in treatment 3 (offered both rotifers and *Artemia*) gradually increased acceptance of *Artemia* as the mean length of larvae increased. A significantly (*P*<0.05) greater percentage of fish with mean lengths ranging from 4.1 \pm 0.3 to 4.9 \pm 0.4 mm, consumed rotifers than *Artemia*. No significant difference (*P*>0.05) in the percentage of fish consuming either rotifers or *Artemia* existed in fish with mean lengths from 5.1 \pm 0.5 to 5.4 \pm 0.4; however a significantly (*P*<0.05) greater percentage of fish consumed *Artemia* than rotifers once the mean length of fish was 5.5 \pm 0.1 mm (Table 3).

The mean number of rotifers and *Artemia* consumed by mulloway larvae was also dependant on fish length. Larvae in Treatment 3 consumed significantly (P < 0.05) more rotifers than *Artemia* until the mean larvae length reached 5.1 ± 0.1 mm, at which time there was no significant difference (P > 0.05) observed between the mean number of rotifers and *Artemia* consumed. From 5.2 ± 0.1 mm larvae began to consume significantly (P < 0.05) more *Artemia* than rotifers. The number (mean \pm SEM) of *Artemia* consumed by larvae in treatment 3 increased steadily from 0.1 ± 0.03 *Artemia* h⁻¹ for larvae of 4.1 ± 0.07 mm, to 13.7 ± 0.31 *Artemia* h⁻¹ for larvae of 5.5 ± 0.1 mm.

Chesson's selectivity index (α) showed significant (P < 0.05) selection for rotifers between 4.1 ± 0.3 to 4.9 ± 0.4 mm. From 5.1 ± 0.5 to 5.4 ± 0.4, there was not a significant difference (P > 0.05) in selection between either rotifers or *Artemia* and neutral selection ($\alpha = 0.5$). Larvae of 5.4 ± 0.1 mm showed significant (P < 0.05) selection for *Artemia* (Figure 4).

3.3 Experiment 3. Mulloway larvae preference selection of Artemia and pellets

The percentage of larvae feeding in treatment 1 (offered only *Artemia*) ranged from 95 ± 0.5 to 100 ± 0.0 % (Table 4). The percentage of larvae feeding in Treatment 2 (offered only pellets) increased throughout the experiment from 25 ± 0.3 to 96 ± 0.5 % of fish with mean lengths 8.3 ± 0.3 to 12.5 ± 0.3 (Table 4). Mulloway larvae in treatment 3 (offered both *Artemia* and pellets) gradually increased acceptance of the pellets as the mean larval length increased. From 8.3 ± 0.3 to 10 ± 0.3 mm, a significantly (P < 0.05) greater percentage of fish consumed *Artemia* than pellets. No significant difference (P > 0.05) in the percentage of fish consuming either *Artemia* or pellets existed for larvae of 10.6 ± 0.4 mm mean length, however a significantly greater (P < 0.05) percentage of fish with mean lengths ranging from 11.6 ± 0.2 to 12 ± 0.4 mm consumed pellets than *Artemia* from (Table 4). This significant difference was not however evident for fish with a mean length of 12.5 ± 0.3 mm.

The mean number of *Artemia* and pellets consumed by mulloway larvae was also dependent on fish length. Larvae in treatment 3 consumed significantly (P < 0.05) more *Artemia* than rotifers until the mean larvae length reached 10.6 ± 0.4 mm, at which time there was no significant difference (P > 0.05) observed between the mean number of *Artemia* and pellets consumed (Figure 5). From a mean length of 11.6 ± 0.2 mm larvae began to consume significantly (P < 0.05) more pellets than *Artemia* (Figure 5). The number (mean \pm SEM) of pellets consumed by larvae in treatment 3 increased steadily from 1 ± 0.2 pellets h⁻¹ for larvae of 8.3 ± 0.3 mm mean length , to 13.5 ± 0.6 pellets h⁻¹ for larvae of 11.6 ± 0.2 mm mean length. Larvae with mean lengths of 12 ± 0.4 and 12.5 ± 0.3 mm did not continue to increase the mean number of pellets consumed, however they did continue to consume significantly more (P < 0.05) pellets than *Artemia* (Figure 5).

Chesson's selectivity index (α) showed significant (P < 0.05) selection for *Artemia* between 8.3 ± 0.3 and 10 ± 0.3 mm. Larvae of 10.6 ± 0.4 mm mean length were observed to have no significant difference (P > 0.05) in selection between either *Artemia* or pellets and neutral selection ($\alpha = 0.5$). Larvae with mean lengths (± SEM) between 11.6 ± 0.2 and 12.5 ± 0.3mm showed significant (P < 0.05) selection for pellets (Figure 6).

In the confirmation of experiment (3), larvae were observed to consume significantly more pellets than *Artemia* once the mean length of fish was between 10 and 11 mm. There was no significant difference observed between the mean number of food items consumed for fish of 8 ± 0.5 to 10 ± 0.5 mm (Figure 7). The percentage of fish that consumed pellets increased linearly (r = 0.936) from 47% in fish with a mean lengths of 8 ± 0.5 mm to 100% in fish with a mean length of 12 ± 0.5 mm. The percentage of fish consuming *Artemia* decreased consistently (r = 0.518) and ranged from 89% in fish with a mean length of 10 ± 0.5 mm to 13% in fish with a mean length of 15 ± 0.5 mm.

4. **DISCUSSION**

This study has determined that mulloway larvae can be weaned directly from rotifers to a pellet microdiet without the use of *Artemia* (Experiment 1); however, this significantly reduces growth. Fish fed *Artemia* in combination with pellets also weaned successfully and displayed better growth rates. The results showed that the weaning process can be achieved more cost effectively by reducing the amount of *Artemia* used by half. This is consistent with other studies which have reported similar results when larvae were not fed *Artemia*. Both Callan et al. (2003) and Fletcher et al. (2007) determined that it was possible to co-feed Atlantic cod (*Gadus morhua*) larvae a microdiet diet along with a reduced amount of *Artemia* without compromising growth and survival, however if *Artemia* was excluded from the diet growth and survival were reduced. Curnow *et al.* (2006) also found that the weaning process for barramundi (*Lates calcarifer*) should include some *Artemia* (5% of previous industry ration) in conjunction with a microdiet in order to stimulate feeding until the stomach is fully developed.

The benefits of incorporating live feeds in the weaning process to increase exogenous enzyme digestion and to stimulate endogenous trypsin secretion in larvae have been discussed previously to explain the importance of co-feeding live feeds with pellet diets (Pedersen & Hjelmeland, 1988; Person Le Ruyet et al., 1993). The reduced growth rates observed when mulloway larvae were not offered *Artemia* in conjunction with pellets, may suggest that larval digestive enzymes were not adequate to digest and assimilate nutrients from the pellet diet. In addition to this, fish in all replicates in the treatments (experiment 1) not offered *Artemia* (3 and 6) were feeding on pellets at mean lengths of 8.8 and 9.6 mm, respectively. These lengths are smaller than those determined to be appropriate to introduce pellet, but larger than was suggested to introduce *Artemia* in feed selection experiments 2 and 3. This may suggest that larvae in these treatments were ready to consume a larger food source, but enzymatic activity was not adequate to effectively digest the pellet. It is also interesting to note that all treatments contained fish that were feeding on pellet once the minimum mean length of 10 mm was attained, which is similar to the outcomes of feed selection experiment 3.

The improved survival of fish fed pellets early (14 dah) rather than later (23 dah) was influenced by the 0 or 50% ration of *Artemia*. The total amount of food in these treatments was lower than in treatments fed a 100% ration of *Artemia*. A reduction in resources or food has been linked with cannibalism (Hecht & Pienaar, 1993), and it is possible that the limit food supply induced cannibalistic behaviour in mulloway which increased mortality. As fish fed pellets from 14 dah did not suffer increased mortality when fed reduced rations of *Artemia*, it is possible that 23 dah was too late for mulloway larvae to be weaned onto pellets.

The transition of food preference from rotifers to *Artemia* occurred once the mean size of mulloway larvae was 5.2 mm, at which time larvae selected significantly more *Artemia* than rotifers. Fish that were only fed *Artemia* consumed them at a greater rate than fish offered both

rotifers and *Artemia*, which indicates that larvae were capable of feeding on *Artemia* but were preferentially selecting rotifers until they reached 5.2mm in length. The selection of smaller prey types can be explained on the basis of energy spent and profits gained. Mayer & Wahl (1997) discussed prey preference of larval walleye (*Stizostedion vitreum*) in terms of profit as the fish selected strongly for prey types that improved growth and survival. Shaw et al. (2003) discussed shifts in prey selection based on prey size and ontogenetic development in greenback flounder (*Rhombosolea tapirina*). Mulloway are likely to have selected rotifers until swimming ability and sensory development had improved enough for it to be advantageous to select the larger, more energy rich *Artemia*.

In Experiment 3, the transition of food preference from *Artemia* to pellets occurred once the size of mulloway was 10.6 mm. The fish that were only fed pellet were found to be consuming them at a much greater rate than fish offered a choice between *Artemia* and pellets. This indicates that although larvae are capable of consuming pellets they were preferentially selecting *Artemia* until reaching a mean length of 10.6 mm. Chesson's selectivity index also indicated that the transition from *Artemia* to pellets occurred at 10.6 mm, after which selection of pellets was significantly greater than neutral selection. Larval studies have found that less digestible prey types are selected against (Olsen et al., 2000), and it is likely that *Artemia* may have been preferentially selected by larvae until reaching a mean length of 10.6mm, as the pellet is more difficult to digest as was discussed for experiment 1. In fish with a mean length of 10.6 mm, digestive capacity may have improved enough for larvae to begin benefiting from energy rich pellets.

The results of the validation trial were similar to that of experiment 3. It was demonstrated that mulloway larvae began to select pellets in preference to *Artemia* once mean larval length was between 10 and 11 mm. The initial experiment measured food preference against the average length of fish in the holding tank, while the validation trial measured food preference against individual larvae lengths. As the results of the two experiments are similar, it suggests that the methodology used in the initial experiment was sufficient to rely on the results obtained using average lengths not individual lengths. The results of the three experiments examining the transition from *Artemia* to pellets were similar. In experiments 1 and 3, and in the validation trial, larvae lengths at the time of pellet selection were 10, 10.6 and 10-11 mm respectively. The similarity in results strongly supports the conclusion that 10.6 mm is an appropriate length to begin weaning larval mulloway from *Artemia* to a pellet microdiet.

The weaning and prey selection studies have shown that the process for weaning larval mulloway needs to include some *Artemia* in conjunction with a pellet microdiet in order for the transition to be successful without compromising growth. It is suggested that the weaning process should begin with rotifers until the mean size of larvae reaches 5.2 mm in length, after which, *Artemia* should be maintained until the mean fish length is 10.6 mm. A 400 µm pellet should then be introduced and the amount of *Artemia* can be gradually reduced over the following days, until all fish are considered to be successfully weaned. These protocols are expected to improve growth rates while reducing costs by minimising the amount of *Artemia* required for weaning and optimising the time of introduction for each new food source.

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Feed treatments applied to fish throughout the feeding trial (n=5 replicates). dah – days after hatching

Treatment	Rotifers	Artemia	Pellet Diet
1	14-29 dah	18-27 dah ^a	14-33 dah
2	14-29 dah	18-27 dah ^b	14-33 dah
3	14-29 dah	Nil	14-33 dah
4	14-29 dah	18-27 dah ^a	23-33 dah
5	14-29 dah	18-27 dah ^b	23-33 dah
6	14-29 dah	Nil	23-33 dah

a = 100% ration, b = 50% ration

TABLE 2

Final weight (dry) and length (mean \pm SEM) with survival (%) for fish for each feeding treatment after 29 days (Experiment 1). A full ration of Artemia began at 0.4/ml per feed at 18 dah and was doubled each day until 21 dah. The ration was then reduced by half each day until 27 dah, which was the last feed of Artemia. The half ration was half of the full ration. dah – days after hatching

Treatment	Artemia (18dah)	Pellet Diet	Weight (mg)	Length (mm)	Survival (%)
1 2 3 4 5 6	Full ration Half ration Nil ration Full ration Half ration Nil ration	14 dah 14 dah 14 dah 23 dah 23 dah 23 dah	$\begin{array}{l} 4.9 \pm 0.5 \\ 4.5 \pm 0.2 \\ ^{b}\\ 1.8 \pm 0.1 \\ ^{a}\\ 4.5 \pm 0.4 \\ ^{b}\\ 4.1 \pm 0.3 \\ ^{b}\\ 2.6 \pm 0.3 \\ ^{a}\end{array}$	$\begin{array}{l} 14.4 \pm 0.6^{b} \\ 14.2 \pm 0.2^{b} \\ 10.0 \pm 0.3^{a} \\ 14.5 \pm 0.5^{b} \\ 13.7 \pm 0.4^{b} \\ 11.6 \pm 0.4^{a} \end{array}$	5.5 ± 3.0^{x} 1.0 ± 0.6^{x} 3.0 ± 1.5^{x} 3.7 ± 1.2^{y} 13.1 ± 4.1^{y} 7.4 ± 3.0^{y}

Different letters in superscript within the same column indicate significant differences (P<0.05; 1-factor ANOVA, P<0.05, SNK).

The percentage (mean \pm SEM; n = 20) of fish feeding on each prey type, in each treatment, on consecutive experiment days. Lengths (mean \pm SEM, n = 20) are those of fish in the holding tank on a given experiment day. Fish in treatment 1 were offered only rotifers (R), those in treatment 2 only Artemia (A), whereas those in treatment 3 were offered both R and A. Treatment 3 is represented by the 3rd and 4th rows as two prey types were available. Different superscripts at the same length indicate significant differences.

Length (mm)	4.1 ± 0.3	4.4 ± 0.4	4.9 ± 0.4	5.1 ± 0.5	5.2 ± 0.5	5.4 ± 0.5	5.5 ± 0.4
Treatment	21 ± 0.8^{ab}	71 ± 1.7^{a}	76 ± 1.1^{a}	88 ± 0.9^{a}	81 ± 0.9^{a}	$88 \pm 1.0^{\mathrm{a}}$	86 ± 0.4^{a}
1 – R							
Treatment	11 ± 0.7^{ac}	67 ± 1.2^{a}	39 ± 1.1^{b}	57 ± 0.7^{b}	59 ± 1.2^{b}	56 ± 0.6^{b}	$81 \pm 0.9a$
2 – A							
Treatment	29 ± 1.0^{b}	58 ± 1.6^{a}	76 ± 1.3^{a}	57 ± 1.4^{b}	$48\pm1.3^{\text{b}}$	46 ± 0.5^{b}	$22\pm0.4^{\text{b}}$
3 – R							
Treatment	$6 \pm 0.5c$	$29\pm0.8^{\text{b}}$	31 ± 1.0^{b}	$49\pm1.5^{\text{b}}$	55 ± 1.2^{b}	57 ± 1.2^{b}	$77 \pm 0.9a$
3 – A							

TABLE 4

The percentage (mean \pm SEM; n = 20) of fish feeding on each prey type, in each treatment, on consecutive experiment days. Lengths (mean \pm SEM, n = 20) are those of fish in the holding tank on a given experiment day. Fish in treatment 1 were offered only Artemia (A), those in treatment 2 only pellets (P), whereas those in treatment 3 were offered both A and P. Treatment 3 is represented in 3rd and 4th rows as two prey types were available. Different superscripts at the same length indicate significant differences.

Length (mm)	8.3 ± 0.3	8.8 ± 0.4	10 ± 0.3	10.6 ± 0.4	11.6 ± 0.2	12 ± 0.4	12.5 ± 0.3
Treatment	95 ± 0.5^{a}	96 ± 0.4^{a}	96 ± 0.4^{a}	98 ± 0.2^{a}	$100 \pm 0.0^{\mathrm{a}}$	98 ± 0.2^{a}	97 ± 0.4^{a}
1 – A							
Treatment	25 ± 0.3^{b}	55 ± 0.7^{b}	70 ± 0.7^{b}	77 ± 1.7^{b}	86 ± 0.9^{b}	81 ± 1.0^{bc}	96 ± 0.5^{a}
2 – P							
Treatment	95 ± 0.0^{a}	94 ± 0.5^{a}	81 ± 0.7^{c}	72 ± 0.9^{b}	$53 \pm 1.1^{\circ}$	78 ± 0.4^{b}	78 ± 0.9^{b}
3 – A		,					
Treatment	8 ± 0.4^{c}	$25 \pm 0.5^{\circ}$	43 ± 1.6^{d}	78 ± 0.5^{b}	80 ± 0.6^{b}	87 ± 0.4^{c}	81 ± 0.8^{b}
3 – P							



Dry weights (mean \pm SEM; n = 5) of fish fed six different feeding regimes (Table 1) throughout the 29 day trial. Different letters indicate significant differences displayed for the 3rd and 4th sample period. T = Treatment, A = Artemia and P = Pellets.



FIGURE 2

Lengths (mean \pm SEM; n = 5) of fish fed six different feeding regimes (Table 1) throughout the 29 day trial. Different superscripts indicate significant differences displayed for the final sampling period only. No significant difference existed any other sample period. T = Treatment, A = Artemia and P = Pellets.



The number of food items (mean \pm SEM; n=20) consumed by fish in one hour, for each treatment, on consecutive days of experiment. Lengths (means \pm SEM, n = 20) are those of fish in the holding tank on a given experiment day. Treatment 1 (only offered rotifers) and Treatment 2 (only offered Artemia) are represented by the bar graph. Treatment 3 (offered both rotifers and Artemia) is represented by the line graph, with each prey type represented by a separate line. Different superscripts between treatment 3 values at the same length indicate significant differences.



FIGURE 4

Selectivity index α (mean \pm SEM) for fish fed a mixed diet containing 50 % rotifers and 50 % Artemia. Fish that did not consume either prey were excluded from the analyses; therefore n range is 3 – 20. Means with a '*' superscript indicate a significant difference from neutral selection.



The number of food items (mean \pm SEM; n=20) consumed by fish in one hour for each treatment, on consecutive days of experiment. Treatment 1 (only offered Artemia) and Treatment 2 (only offered rotifers) are represented by the bar graph. Treatment 3 (offered both rotifers and Artemia) is represented by the line graph, with each prey type represented by a separate line. Different superscripts between treatment 3 values at the same length indicate significant differences.



FIGURE 6

Selectivity index α (mean \pm SEM) for fish fed a mixed diet containing 50% Artemia and 50 % pellet microdiet. Fish that did not consume either prey were excluded from the analyses; therefore n range is 17 – 19. Means with a '*' superscript indicate a significant difference from neutral selection.



The number of food items (mean \pm SEM; n = 11-50) consumed by fish after one hour of feeding in the validation trial. Means are pooled for the two replicate tanks. Different superscripts at the same time period indicate significant differences (*P*<0.05). Lengths have been rounded to the nearest mm.

4.4 Photoperiod and feeding interval requirements of juvenile mulloway, *Argyrosomus japonicus*

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ABSTRACT

Two experiments were completed to determine the most efficient feeding interval and photoperiod for rearing juvenile mulloway, Argyrosomus japonicus. The first, a two factor experiment incorporating three photoperiods (12L:12D, 18L:6D and 24L:0D), and five feeding intervals (1, 3, 6, 12 and 24 h between feeds), examined the effects on somatic growth rate, feeding efficiency and survival of juvenile mulloway (2.7 g \pm 0.5 g). The growth indices tested included fish weight, length, food conversion ratio - FCR, condition factor - CF, and the coefficient of variation of weight - CVwt. No interactions were found for any variables measured with the result that photoperiod and feeding interval outcomes were discussed separately. The 12L:12D photoperiod produced significantly poorer survival than other photoperiods, however feeding interval did not influence survival. The 24L:0D photoperiod led to significantly reduced growth rates, compared to that of 12L:12D and 18L:6D. The CF of the 12L:12D photoperiod was reduced compared to other photoperiods as the fish were significantly longer in comparison to their weight, however the FCR of the 12L:12D photoperiod was significantly better than the other photoperiods. The 24 h feeding interval resulted in reduced lengths and weights than fish from other feeding intervals, however no differences occurred between the 1, 3, 6 and 12 h feeding intervals. The CVwt was not affected by either photoperiod or feeding interval. The second experiment assessed gastric evacuation by juvenile mulloway after being fed once to satiation. Stomach contents were evacuated at a steady rate with a 50% reduction in stomach fullness after 10 h and 100% evacuation of stomach contents after 20 h. These experiments suggest that a photoperiod of 18L:6D and a 12 h feeding interval is the most efficient way of producing high growth rates and survival for juvenile mulloway.

1. INTRODUCTION

Mulloway (*Argyrosomus japonicus*) are common fish in Australia's south-eastern waters (except Tasmania) with a distribution stretching from Exmouth in Western Australia to Brisbane in Queensland (Edgar, 2000). Mulloway are commonly encountered in marine and estuarine environments and juveniles can often be found in the upper reaches of estuaries (Gray & McDonall, 1993). Mulloway are a popular table fish fetching a high price and are targeted by both commercial and recreational fishers. Due to the high demand for mulloway, they have been recognized as an important aquaculture species in Australia (Battaglene & Talbot, 1994; Fielder, Bardsley & Allan, 1999).

As with many new aquaculture species in Australia, mulloway production is currently restricted by high costs. In order to improve production efficiency in hatcheries it is important to optimize the conditions in which the fish are reared. These conditions can include the physical culture environment (temperature, salinity, light intensity and photoperiod) (Black, 1998) and general nutritional parameters such as diet composition, ration and feeding frequency (Jobling, 1998).

The benefits of photoperiod manipulation to improve growth rates have been well documented. Boeuf and Le Bail (1999) suggested that day length or photoperiod may indirectly modify growth by increasing food intake and/or muscle mass by exercise. Red sea bream (*Pagrus major*) and largemouth bass (*Micropterus salmoides*) have displayed a higher weight gain and specific growth

rate in longer photoperiods (Petit, Beauchaud, Attia & Buisson, 2003; Biswas, Seoka, Inoue, Takii & Kumai, 2005), which have been primarily attributed to an increase in the length of feeding time available and a corresponding increase in the food consumed (Sayer, 1998; Petit et al., 2003). Alternatively by increasing the length of feeding time, energy expenditure may increase with the result that some species have reduced growth rates (Fielder, Bardsley, Allan & Pankhurst, 2002; Biswas and Takeuchi, 2003). This was evident in the work conducted by Ginés, Afonso, Arguello, Zamorano & Lopez (2004), who found that the growth rate of immature gilthead sea bream (*Sparus aurata*) was reduced when held under continuous light even though food consumption was greater than other photoperiods.

Manipulation of photoperiod can also affect feed conversion ratios or efficiencies and the condition factor of fish (Boeuf & Le Bail, 1999; Biswas et al., 2005), and can be largely species specific, demonstrating the importance of investigating the effect photoperiod has on growth and feeding efficiency of each new aquaculture species. Better food conversion efficiencies were achieved in *M. salmoides* under continuous light when compared to a 12L:12D photoperiod (Petit et al., 2003), and the growth of *P. major* was enhanced by improved food conversion efficiencies in longer photoperiods (Biswas et al., 2005). In contrast, Biswas and Takeuchi (2003) found longer photoperiods resulted in reduced condition in Nile Tilapia (*Oreochromis niloticus* L.), while Barlow, Pearce, Rodgers & Clayton (1995) reported no difference in condition of juvenile barramundi (*Lates calcarifer*) when reared in 12 or 24 hours of light.

Feeding frequency also affects growth rates and can influence the total mass gained and specific growth rates (SGR) of juvenile fish (Reddy & Leatherland, 2003). The optimal feeding frequency may be directly related to feeding efficiency as appropriately spaced feeds may better allow food to be ingested, digested and assimilated (Biswas & Takeuchi, 2003). A review of optimal feeding frequencies for a range of species completed by Zhou, Cui, Xie, Zhu, Lei, Xue & Yang (2003) highlights the variability in feeding habits for different species and reported that differences can be the result of different feeding behaviour or stomach capacity. In addition, the most efficient feeding interval is also likely to be influenced by the gastric evacuation rate of the species and how quickly appetite will return after feeding (Smith, 1989). Appetite is strongly dependent on stomach fullness, and while food is being digested and evacuated from the stomach, fish become increasingly motivated to feed (Olsen & Balchen, 1992).

A change in feeding interval can affect food consumption, food conversion ratios (FCR) and the coefficient of variation for weight (CVwt) (Dwyer, Brown, Parrish & Lall, 2002). Wang, Hayward and Noltie (1998) observed that more frequent feeding reduced the size variation of hybrid sunfish larvae. A significant variation of weight within a population can lead to hierarchical feeding patterns and thus may further influence the growth performance of individuals having different social status (Sayer, 1998). Cannibalism within a cohort of fish is also facilitated by size variation (Kestemont, Jourdan, Houbart, Melard, Paspatis, Fontaine, Cuvier, Kentouri & Baras, 2003), with the result that mortality rate within the population will increase.

The combined effects of photoperiod and feeding interval have been investigated on species such as Nile tilapia (*Oreochromis niloticus*) and snapper (*Pagrus auratus*) with optimal combinations resulting in significantly improved growth rates (Biswas & Takeuchi, 2003; Tucker, Booth, Allan, Booth & Fielder, 2006). Initially this study investigated the interactive effects of a range of feeding intervals and photoperiods on the somatic growth rate and feeding efficiency of juvenile mulloway as assessed by change in body weight and length, FCR, CF, CVwt and survival. We then assessed the rate of gastric evacuation after one feed as measured by change in stomach fullness as a percentage of body weight over time.

2. MATERIALS AND METHODS

2.1 Fish and facilities

Juvenile mulloway, 2.7 g \pm 0.5 g (mean \pm SEM), reared at the Port Stephens Fisheries Institute, NSW, Australia, were stocked into 60 l (700 X 360 X 238 mm) clear acrylic experiment tanks. Fish used in Experiments 1 and 2 were from different cohorts of fish. A clear perspex lid covered each tank and the light intensity (18W fluorescent tubes, Davis) at the air-water interface was 1-2 μ mole s⁻¹ m⁻² during the light phase (LI-COR, model Li-1776, USA). Lights were set to turn on and off immediately, without a dimming effect. Tank effluent water was collected in a 1000 l sump, pumped through 25 μ m cartridge filters into a trickle biofilter and then recirculated to tanks at a flow rate of 0.5 l min⁻¹. Approximately 150% of the total system seawater was exchanged daily.

For Experiment 1, banks of tanks were divided into three photoperiod treatment bays; each bay separated with black plastic sheeting to exclude light leakage between adjacent photoperiod treatments.

2.2 Experiment 1: The effects of photoperiod and feeding frequency on juvenile mulloway

A two-factor experiment was conducted over 30 days at the PSFI to determine the interactive effects of feeding interval and photoperiod on the growth and feeding efficiency of juvenile mulloway.

Five feeding intervals were investigated (one feed every 1, 3, 6, 12 or 24h) across three photoperiods (24L:0D, 18L:6D and 12L:12D). Each treatment combination was replicated four times with each replicate tank containing 20 fish. Individual treatment combinations are expressed as photoperiod and feeding interval (light: dark, hours between feeds e.g. 12L:12D; 12 h represents 12L:12D photoperiod; 12 h feeding interval).

All fish were fed 10% of their body weight daily (Ridley Aqua-feed, Narangba, Australia; Native fish starter: Crude Protein - 52%; Crude Fat - 12%; Ash - 13%; Fibre - 3%), which exceeded the total intake, to ensure feed availability did not limit growth. Each day the total food for individual tanks was divided evenly across the number of feeds assigned to that tank. Uneaten feed was removed by siphon daily and was pooled for each tank in ten day batches to be dried (16 h at 105°C) and weighed. The total biomass (g) of fish in each tank was measured every ten days and feed weights were adjusted accordingly. Weights of feed delivered per feeding episode and weights of uneaten feed were used to calculate feed intake as a % of body weight/feeding episode. Fish were given 1- h prophylactic formalin treatments (200ppm) on the day of each weight check to reduce the risk of infection by the dinoflagellate ectoparasite *Amyloodinium ocellatum*. Fish in all treatments were fed a reduced ration of 1% body weight in one feed on these occasions as the feeding schedule was interrupted.

For the 12L:12D and 18L:6D photoperiod treatments, lights were set to turn on at 0730 h and the first feed addition for all photoperiods was at 0800 h. The 12L:12D, 12 h treatment did not enable enough hours of light to complete the two assigned feeds during the light phase. Therefore, the second feed was provided in the dark phase. Fish in the 12L:12D, 24 h treatment were fed just once during the light phase and a subsequent comparison of weight gain between this and the 12L:12D, 12 h treatment, may provide supporting evidence as to whether the fish fed in darkness.

Upon completion of the experiment the final percent survival within each tank and weight and length measurements of all fish were obtained. Feeding indices used to assess performance were: Feed conversion ratio (FCR) = Feed (dry weight)/ fish weight gain (wet weight) (Hardy, 1989); Condition factor (CF) = 100^{*} (Weight_(g)/Length_(cm)^b), where b is estimated by the length-weight relationship equation (W = aL^b) of the sampled populations (King, 1995); Coefficient of Variation of weight (CV_w) = (s/mean) x 100, where s is the standard deviation.

2.3 Experiment 2: Gastric evacuation of juvenile mulloway

A gastric evacuation experiment was conducted to determine the time taken for juvenile mulloway to evacuate an entire feed when fed to satiation. Fifty seven experiment tanks were each stocked with 15 juvenile mulloway raised from a separate cohort of fish and were acclimated to a feed regime of one feed at 0900 h for three consecutive days. Three randomly selected tanks were euthanased using a lethal dose of ethyl-p-amino-benzoate (100mg/l) at 0900 h on the day of the trial and were examined to ensure the fish stomachs were empty. The remaining treatment tanks were fed to satiation at 0900 h and fish from three randomly selected tanks were euthanased every 2 h for 36 h. Euthanased fish were immediately frozen for later dissection and analysis of stomach contents.

A sub sample of five frozen fish was randomly selected from each replicate tank and thawed. The stomach from the cardiac to pyloric sphincter was dissected from each fish and the content of each stomach was then removed. The remaining whole fish and stomach content were dried at 105°C for 16 h. The dry weights of the fish and its stomach content were obtained and these data were used to determine the stomach fullness as a percentage of total body weight = [dry wt (g) of stomach contents/ dry wt (fish-stomach contents)]*100.

2.4 Water quality analyses

Water quality (mean \pm SD) was measured daily using a water quality meter (Horiba U-10, Japan): pH (7.77 \pm 0.14), dissolved oxygen (5.99 \pm 0.22 mg l⁻¹), temperature (21.9 \pm 1.80°C), and salinity (31.2 \pm 1.77‰). Total ammonium (NH₄⁺) (0.31 \pm 0.13 mg l⁻¹) was measured each day using a rapid test kit (E.Merck, Model 1.08024, Germany). Water quality parameters were consistent across all treatment tanks for the duration of the experiments.

2.5 Statistical analyses

Data in experiment 1 were assessed for homogeneity of variance using Cochran's test (Underwood, 1997). The experiment was designed for analysis by two-factor analysis of variance (ANOVA) to determine the effects of feeding frequency and photoperiod, and their interaction, on growth, FCR, CF, CVwt and survival. Where significant differences were found, the means were compared by the Student – Newman - Keuls test (SNK). Statistical analyses were conducted using Statgraphics Version 4.1 (STSC, USA).

3. **RESULTS**

3.1 *Experiment 1: The effects of photoperiod and feeding frequency on juvenile mulloway*

There were no significant interactions (P>0.05) between photoperiod and feeding interval for all variables measured, therefore, photoperiod and feeding interval outcomes were discussed separately (Table 1). The length and weight of fish increased in all experimental treatments regardless of feeding interval or photoperiod.

Photoperiod had a significant effect (P>0.05) on the weight of fish, with fish in the 12L:12D (7.4 ± 0.2g) and 18L:6D (7.5 ± 0.2g) photoperiods heavier after the 30 day trial than fish in the 24L:0D (6.6 ± 0.2g) photoperiod (Figure 1, Table 2). Photoperiod also influenced fish length (mean ± SEM; n=20), with fish cultured in the 12L:12D photoperiod longest (8.7 ± 0.2 cm), followed by the 18L:6D photoperiod (8.4 ± 0.1 cm), and the 24L:0D photoperiod (7.9 ± 0.1 cm) (Table 2).

The FCR of fish was significantly affected (P<0.05) by photoperiod. Fish exposed to the 24L:0D photoperiod displayed a higher FCR than those reared in the 18L:6D photoperiod, while the 12L:12D photoperiod had the lowest FCR. The CF of mulloway was also affected by photoperiod with the CF of fish reared in 12L:12D lower than those reared in 18L:6D and 24L:0D (Table 2). The lower CF for the 12L:12D photoperiod was a result of significantly longer but not heavier individuals in comparison with the 18L:6D and 24L:0D photoperiods as previously described.

Photoperiod did not affect CVwt (P>0.05) (Table 2). Average feed consumption per day was significantly higher in the 18L:6D ($4.0 \pm 0.13g$) and 12L:12D ($3.9 \pm 0.12g$) photoperiods compared to the 24L:0D ($3.1 \pm 0.11g$) photoperiod. The increase in feed consumption was a result of better growth of fish in these photoperiods (weight and length; Table 2).

Survival of fish held in 12L:12D (93.3 \pm 1.6%) was significantly lower (P<0.05) than that of fish held in 18L:6D (98.3 \pm 0.5%) and 24L:0D (97.5 \pm 1.5%). No significant difference (P>0.05) in survival of fish was found between the feeding intervals.

Feeding interval had a significant effect on the weight and length of fish (Figure 2). Mulloway cultured with a 24h feeding interval were significantly (P<0.05) lighter and shorter than fish in all other feeding intervals (Table 2). The percentage body weight consumed per feed increased linearly ($R^2 = 0.9992$) from the 1h feeding interval to the 12h feeding interval (Figure 3). When the 24 h feeding interval is included this linear trend is not as strong (R^2 =0.9838) as these fish consumed an average of more than twice that of fish held in the 12 h feeding interval (feed consumed % B.W feed ⁻¹) (Figure 3). However, the total weight of the feed consumed for this 24h feeding interval was less than other feeding intervals as a result of smaller fish weights (Table 2).

The FCR, CF and CVwt were not significantly affected (P>0.05) by feeding interval over the thirty day experiment (Table 2), however the FCR did vary significantly for each ten day weight period. From day 0 to day 10 the FCR for the 12h feeding interval was higher than the 1h, 3h and 6h feeding intervals and the FCR for the 24h feeding interval was higher than all other feeding intervals. From day 11 to day 20 there was no difference in FCR between the feeding intervals but from day 21 to day 30 the FCR successively increased as feeding interval was increased. The final result of this change in FCR is that no difference was found between feeding intervals for the average FCR over thirty days (Table 2).

The fish in the treatment 12L:12D, 12h were fed once under light and once in darkness. The mean weights (mean \pm SEM; n=4) of the fish in this treatment (7.8 \pm 0.4g) were higher than that of the treatment 12L:12D, 24h (6.3 \pm 0.2g), which were only fed once in the light.

3.2 Experiment 2: Gastric evacuation of juvenile mulloway

The amount of food remaining in the stomach of juvenile mulloway after a single feed to satiation decreased steadily over a 20h period (Figure 4). At 10 hours after feeding, approximately 50% of the food consumed had been evacuated from the stomach. The linear relationship between 2h and 20h after feeding (R^2 =0.9477), can be expressed by y = -0.3417x + 6.8101.

4. **DISCUSSION**

This study has established that there are no interactive effects of photoperiod and feeding interval on juvenile mulloway, which differs from a previous study on juvenile snapper (*P. auratus*), another temperate marine species (Tucker et al., 2006).

Experiment 1 determined that photoperiod can have a significant influence on the survival and growth of juvenile mulloway. Under a 24L:0D photoperiod, significantly poorer growth (weight, length and FCR) of mulloway was obtained compared with that of fish reared under 12L:12D and 18L:6D photoperiods. This is consistent with previous research that demonstrated optimizing photoperiod regimes is necessary to obtain high growth rates (Boeuf & Le Bail, 1999). Biswas & Takeuchi (2003) found that a 24L:0D photoperiod is less effective in obtaining high growth rates (in terms of Specific Growth Rate - SGR) for Nile tilapia (*Oreochromis niloticus*) when compared to 3L:3D, 6L:6D and 12:L12D photoperiods. These authors attributed the respective SGR values to the balance between energy intake and energy expenditure at the different photoperiod treatments. A continuous light regime is likely to require greater energy expenditure associated with increased swimming activity than shorter light regimes and may have contributed to significantly higher FCR and reduced growth rates for mulloway. In contrast to this, a continuous photoperiod has been associated with improved growth rates for some species as the length of feeding time available to

fish is increased (Sayer, 1998). Tripple & Neil (2003) found an increase in the weight of juvenile haddock (*Melanogrammus aeglefinus*) reared under continuous light for 24 weeks compared to fish reared under natural photoperiods. Simensen, Jonassen, Imsland and Stefansson. (2000) also found improved SGR in Atlantic halibut (*Hippoglossus hippoglossus*) when reared under continuous light compared to fish reared under shorter photoperiods. As the results of photoperiod trials on fish growth have been inconsistent between species, it is apparent that photoperiod requirements are species specific and growth rates may partly depend on the species ability to reduce energy expenditure in long light phases.

The FCRs for juvenile mulloway were observed to increase with day length. The 12L:12D photoperiod produced the lowest FCR, while the 24L:0D photoperiod had the highest FCR. This suggests that juvenile mulloway are better at converting energy into somatic growth during shorter photoperiods. In contrast to this, Rad, Bozaoglu, Gozukara, Karahan and Kurt (2006) found that continuous light improved the FCR of *O. niloticus*, again indicating that growth performance under long photoperiods is species specific. Mulloway held in the long light phase also consumed less food per day than the shorter photoperiods, even though there was more light available for feeding. This is likely to have been influenced by the poorer FCRs, which contributed to smaller fish unable to consume as much as the larger fish from the other photoperiods.

In addition to the poorer FCRs observed in juvenile mulloway during long light phases, it appears that fish length is inversely related to day length. Melatonin levels are also inversely related to day length (Randall, Bromage, Thorpe, Miles & Muir, 1995), and melatonin along with the pineal gland, have previously been reported to influence skeletal growth in fish (Fjelldal, Grotmol, Kryvi, Gjerdet, Taranger, Hansen, Porter & Totland, 2004). It is well documented that melatonin levels are higher in fish during darkness (Randall et al., 1995; Porter, Randall & Bromage, 1996; Bayarri, Rodriguez, Zanuy, Madrid, Sanchez-Vazquez & Carrillo, 2003) and this may have contributed to longer fish lengths in the treatments that had longer dark phases. The differences found in fish length also contributed to lower CF in the 12L:12D photoperiod, as these fish were longer but not heavier than fish in the 18L:6D photoperiod. CF is said to be an indicator of the well being of fish (Bolger & Connolly, 1989) and although the fish were longer and displayed improved FCRs, the reduced CF may have contributed to the increased mortality rate observed in the 12L:12D photoperiod.

In the 12L:12D; 12h treatment fish were fed once in the light and once in darkness. The weights of these fish were heavier than that of fish fed only once in the light (12L:12D; 24h), providing evidence that fish may have been feeding in the dark by non visual means. This is similar to results of Harpaz, Hakim, Barki, Karplus, Slosman & Eroldogan (2005), who found that juvenile *L. calcarifer*, consumed food supplied in the light or dark. Sayer (1998) described how the incidence of nocturnal feeding can increase in winter when the photophase is shorter as there is less opportunity for daylight feeding. It may be useful to study this aspect of mulloway culture further to determine if additional gains in growth performance indices and survival could be obtained by night feeding in conjunction with daytime feeding under a range of short and long photoperiods.

Feeding interval also has a significant effect on the growth and feeding efficiency of juvenile mulloway. While there was no significant difference between the 1h, 3h, 6h and 12h feeding intervals, the 24h interval resulted in poorer growth. This is similar to results of Dwyer et al. (2002) who found that feeding yellowtail flounder (*Limanda ferruginea*) two or four daily meals produced better growth than fish fed once daily, attributing this to reduced food consumption in low feeding frequencies. The fish in the 24h feeding interval did consume less feed than other feeding intervals but this was confounded by the poor FCRs in the first 10 days, which led to slow growth and smaller fish. Over time the FCRs improved and the percent body weight consumed per feed actually became greater than that of the other feeding intervals. While the results of this study indicate that one feed per day is not a suitable feeding regime for juvenile mulloway, it is possible that fish may adapt to the regime over time and growth rates may improve.

Gastric evacuation rate and meal size are major factors affecting the post-prandial return of appetite and subsequent growth, with different species returning to feed after varying amounts of stomach evacuation (Smith, 1989). Species such as rainbow trout (*Oncorhynchus mykiss*) and squawfish (*Ptychocheilus oregonensis*) only feed once their stomach is near to empty, while juvenile pink salmon can return to feeding after as little as 15% of their stomach contents have been evacuated (Smith, 1989). Consequently, the difference in gastric evacuation and voluntary return to feeding between species greatly influences the optimal feeding regime.

The study on gastric evacuation of juvenile mulloway has shown that it can take up to 10 h before 50% of the food consumed in a single meal was evacuated from the stomach. The linear relationship between feed interval (h) and food intake (% B.W. Feed⁻¹) suggests that juvenile mulloway will continue to eat throughout the day but will eat larger amounts as the feeding frequency decreases, with the result that total food consumption does not increase appreciably with feeding frequency.

In conclusion growth rates and efficient feeding can be optimised by rearing juvenile mulloway in an 18L:6D photoperiod and by feeding once every 12 hours. The 24L:0D photoperiod produced poor growth (weight, length and FCR) compared to the 18L:6D and 12L:12D photoperiods and the 12L:12D photoperiod had reduced survival compared with the other photoperiods with the result that the fish in the 18L:6D photoperiod had optimal growth and survival parameters. The only feeding interval to have an effect on growth (weight and length) was the 24 h feeding interval, with no difference in growth detected between the 1 h through to the 12 h feeding intervals. It was also determined that it would take 10 h for 50% of the feed consumed in one meal to be evacuated from the stomach. Given that there was no significant difference in growth and feeding efficiency in juvenile mulloway between the 1h, 3h, 6h and 12h feeding intervals, we suggest that it would be more efficient for producers to feed their fish no more than twice daily (or every 12 h).

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ANOVA results (P-value) for each parameter (Weight, Length, FCR, CF and CVwt) and factor (Photoperiod, Feeding Interval and Interaction)

Parameter	Photoperiod	Feeding Interval	Interaction
Weight	< 0.0001	<0.0001	0.1453
Length	< 0.0001	< 0.0001	0.1716
FCR	< 0.0001	0.3014	0.0860
CF	< 0.0001	0.1379	0.2307
CVwt	0.5359	0.1299	0.9415

Weight, length (total length), feed conversion ratio (FCR), condition factor (CF), co-efficient of variation for weight (CVwt) of mulloway from varying photoperiods and feeding intervals 1, 2

Main effect	Level	Weight (g)	Length (cm)	FCR	CF	CVwt
Photoperiod (b)	12	7.4 ± 0.3^{a}	8.7 ± 0.2^{a}	1.1 ± 0.1^{a}	5.8 ± 0.1^{a}	0.2 ± 0.0
(11)	24	7.5 ± 0.3 6.6 ± 0.3 ^b	8.4 ± 0.1 7.9 ± 0.1 ^c	1.2 ± 0.0 $1.5 \pm 0.1^{\circ}$	6.4 ± 0.1 6.4 ± 0.1 ^b	0.2 ± 0.0 0.2 ± 0.0
Feed interval	1	7.4 ± 0.3 ^x	8.4 ± 0.3 ^x	1.2 ± 0.0	6.2 ± 0.1	0.2 ± 0.0
(hrs b/w feeds)	3	7.6 ± 0.5 ^x	8.5 ± 0.3 ^x	1.3 ± 0.1	6.3 ± 0.1	0.2 ± 0.0
	6	7.6 ± 0.2 ^x	8.5 ± 0.2 ^x	1.3 ± 0.1	6.3 ± 0.1	0.2 ± 0.0
	12	7.3 ± 0.3 ^x	8.4 ± 0.2 ^x	1.3 ± 0.1	6.1 ± 0.1	0.2 ± 0.0
	24	6.1 ± 0.3 ^y	$7.8\pm0.3~^{\rm y}$	1.3 ± 0.0	6.1 ± 0.1	0.2 ± 0.0

¹ Within each factor and column, values (level means \pm SEM; n=20 for photoperiod, n=12 for feeding interval) with

the same letter in the superscript are not significantly different (P>0.05; two-way ANOVA, SNK)

² There were no significant interactions (\tilde{P} >0.05) between photoperiod and feeding interval for any of the growth indices.

Feed conversion ratio (FCR) = Feed (dry weight)/ fish weight gain (wet weight) (Hardy, 1989); Condition factor (CF) = $100*(Weight_{(g)}/Length_{(cm)}^{b})$, where b is estimated by the length-weight relationship equation (W = aL^{b}) of the sampled populations (King, 1995).

Coefficient of Variation of weight $(CV_w) = (s/mean) \times 100$, where s is the standard deviation



Mean \pm SEM (n=12) wet weights of juvenile mulloway held under three different photoperiods; 12 h of light, 18 h of light and 24 h of light. Feeding Interval data is pooled. Mean \pm SEM values with different superscripts at the same time period indicate significant differences.



FIGURE 2

Mean \pm SEM (n=12) wet weight of juvenile mulloway reared on different feeding intervals over an experiment duration of 30 days. Durations between feeds were 1 h, 3 h, 6 h, 12 h and 24 h. Photoperiod data is pooled. Mean \pm SEM values with different superscripts at the same time period indicate significant differences.

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Mean \pm SEM (n=12) percentage of body weight (g) consumed per feed for different feeding intervals over the 30 day trial. Feeding intervals were 1 h, 3 h, 6 h, 12 h and 24 h between feeds. Photoperiod data is pooled. Trend lines: — 1 to 24 h feeding intervals, ---1 to 12 h feeding intervals.



FIGURE 4

Change in stomach fullness assessed as the mean \pm SEM (n=15) weight of the stomach content as a percentage of fish body weight (g) of juvenile mulloway over time (post-prandial hours) after being fed once to satiation. All weights used in calculations are dry weights.
4.5 Changes in feeding behaviour and development of the sensory organs of larval and juvenile mulloway, *Argyrosomus japonicus*

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1. INTRODUCTION

Australian marine finfish larvae invariably hatch very early in development from small sized eggs (Battaglene & Talbot, 1994; Cobcroft & Pankhurst, 2003). Larval development is rapid after hatching and ensures the morphological features required by larvae to undertake feeding activities are present once the yolk has been depleted. Vision, mechanoreception and chemoreception are the three major sensory systems employed by fish for detecting prey and evading predators.

For many species of larvae, vision is the primary sense mode for feeding and the eyes are generally pigmented and considered functional by first feeding (Blaxter, 1988). As larval ontogeny proceeds, mechanoreception and chemoreception can play an increasingly important role in feeding. Larvae typically hatch with a few superficial neuromasts around the head and along the trunk (Cobcroft & Pankhurst, 2003). These superficial neuromasts proliferate after hatching and increase in size, allowing the larvae to detect hydrodynamic disturbances created by prey organisms and can sometimes allow larvae to feed in dark environments (Pankhurst, 2008). The chemosensory organs or olfactory organs can vary in morphology and depend on the degree of development of the species olfactory system (Hara, 1971).

To examine the sensory basis for prey detection, fish fed in the light and dark can be compared for feeding ability to determine the instance of visual and non-visual feeding (Pankhurst, 2008). To further examine the feeding modality in non-visual feeders, the aminoglycoside antibiotic, streptomycin sulphate, can be used to pharmacologically ablate the superficial neuromasts of fish, thus blocking the mechanosesory role and leaving only chemoreception (and inner-ear mechanoreception) (Pankhurst, 2008).

The feeding behaviour of larval mulloway, *Argyrosomus japonicus*, has been studied before and it was determined that larvae are visual feeders from first feeding but later develop the ability to feed in the dark (Ch 4.2). Mulloway are typically thought to spawn in nearshore coastal waters where eggs and larvae are dispersed in the surrounding estuarine and coastal waters and are then recruited to the upper reaches of estuaries as juveniles (Silberschneider & Gray, 2008). This study aimed to determine the feeding modalities employed by larval and juvenile mulloway as ontogeny proceeds and was designed as a multi-factor experiment, in which larval and juvenile mulloway were fed in light and dark conditions to determine the role of vision in feeding, while ablation of the neuromasts using streptomycin sulphate (Jones & Janssen, 1992) enabled the role of mecanoreception and chemoreception in non-visual feeding to be determined.

2. MATERIALS AND METHODS

2.1 Source of larvae

Mulloway broodstock held at the Port Stephens Fisheries Institute (PSFI) were induced to spawn using temperature cues in September 2007. Fertilised eggs were collected, treated with ozone (CT = 0.5) and transferred to an 8000 l clear-water holding tank. The temperature in the holding tank was maintained at 20-22°C, and larvae were fed rotifers at 5/ml from 2.5 mm (first feeding) until 5.5 mm, and then *Artemia* at 0.5/ml until 10.6 mm, when they were weaned onto a 400µm pellet. The tank water was exchanged at a rate of 5 l/min, which ensured clearance of live feeds from the tank during the night. Twenty fish were sampled from the holding tank on each day of the experiment, and prior to the addition of food in the tank, to obtain the mean total length of larvae and to confirm that no residual food remained in the digestive tract from the previous day. Larvae were maintained in ambient light conditions.

2.2 Ablation of the lateral line in mulloway using streptomycin sulphate (SS)

An experiment was completed to determine the efficacy of Streptomycin Sulphate (SS) at 4 concentrations (0.0, 0.5, 0.75 and 1.0 g/l) to ablate the mechanoreceptive neuromasts of larval mulloway (Reference). Twenty larvae (3.5 mm) for each treatment replicate (n = 3) were transferred in a fine mesh dip net from the holding tank to a solution of SS. Larvae were treated in their respective SS solution for three hours, after which they were placed into a Petri dish of sterilised estuarine water. All white light was excluded from the room and larvae were examined under red lights using a dissection microscope. Larvae were approached with a blunt probe and the presence or absence of a startle response was recorded for each fish.

2.3 Feeding ability of fish treated with streptomycin sulphate (SS) and salt water (SW)

An experiment was conducted to determine the effects of SS ablation of the lateral line on the feeding success of larvae offered food in both light and dark conditions. There were four treatments: Treatment 1, SS treated and fed in the dark (only chemoreception available); Treatment 2, SS treated and fed in the light (chemoreception and vision available); Treatment 3, Saltwater (SW) treated and fed in the dark (chemoreception and mechanoreception available); and Treatment 4, SW treated and fed in the light (all senses available).

The experiment began when fish were a mean length of 3.5 mm and was repeated at regular intervals until fish reached a mean length of 53.3 mm. Experiment fish were transferred before feeding on the morning of each experiment day from the holding tank to a treatment bath. One treatment bath contained 0.75g/l of SS and the other was a control bath and contained only sterilised salt water. Fish remained in the treatment bath for three hours and then 30 fish were transferred to each 10 l blue feeding vessel and placed in an individual experiment enclosure. Fish were fed a food type appropriate to their size and started with rotifers (*Brachionus plicitilis*) until fish reached a mean length of 5.5 mm, then *Artemia* until 10.6 mm and were then offered an appropriately sized pellet diet for the duration of the experiment. Each experiment replicate was left to feed in either light (400 lux) or dark (0 lux) conditions for 1 h, after which, fish were euthanased with 100ppm Aqui – S[®] and 20 of the 30 fish were randomly selected and placed on a cold slide, secured with a cold cover slip and examined immediately under a dissection microscope for the number of fish that had consumed food in 1 h and the number of food items in each individual stomach. Feeding treatments were staggered at 30 minute intervals to enable each treatment to be harvested and examined immediately after the tank was euthanased.

SS treated larvae were periodically checked throughout the feeding trial for a 'startle' response when approached with a blunt probe under red light conditions to ensure the concentration of SS remained adequate for lateral line ablation.

2.4 Statistical analysis

Data were assessed for homogeneity of variance using Cochran's test. When variances were heterogeneous, data were log transformed to establish homogeneity. One-factor ANOVA was then used to determine the effect of treatment on feeding intensity and the number of larvae feeding. Where statistical differences were found, means were compared using the Student-Newman-Keuls test.

3. **RESULTS**

3.1 Ablation of the lateral line in mulloway using streptomycin sulphate

Larvae treated with SS at 0.5g/l elicited a 'startle' response 15% of the time when approached with a blunt probe under red light conditions. The reactions of the fish were obvious and immediate. Larvae treated with SS at 0.75g/l reacted 10% of the time, however reactions were delayed and not immediate. Larvae treated with 1g/l SS displayed no reaction to a blunt probe however larvae also appeared disoriented and did not react to a probe when lightly touched on the side flanks. Therefore 0.75g/l SS appears to be the most suitable for ablating the lateral line in mulloway larvae as any response when approached by a blunt probe was not immediate but larvae appeared to still behave normally.

3.2 Feeding ability of streptomycin sulphate and salt water treated mulloway

3.2.1 Live feeds

Fish consumed significantly more (P < 0.05) food items (rotifers and *Artemia*) from 3.5 to 5 mm when fish were fed in light conditions than in dark condictions. From 5.8 mm to 10 mm, the number of food items consumed by fish was lower when chemoreception (and inner ear mechanoreception) was the only sensory system available, while the number of food items was significantly greater (P < 0.05) when vision and mechanoreception were available.

A significantly greater proportion (P < 0.05) of fish were feeding (rotifers and *Artemia*) from 3.5 to 4.1 mm when vision was available in light conditions (Figure 2). From 5 mm to 7.5 mm (but excluding fish of 5.8 mm), the proportion of fish feeding was greater when vision and mechanoreception were available than for fish with only chemoreception (and inner ear mechanoreception). From 8.7 to 10 mm, there was no significant (P > 0.05) differences in the number of fish feeding in each treatment (Figure 2).

3.2.2 Pellet diets

Fish consumed significantly more (P < 0.05) food items (pellets) from 10.6 to 16 mm when vision was available in light conditions (Figure 3). From 22.5 to 53.3 mm, the number of food items consumed by fish was lower when chemoreception (and inner ear mechanoreception) was the only sensory system available, while the number of food items was significantly greater(P < 0.05) when vision and mechanoreception were available (Figure 3).

A significantly greater proportion (P<0.05) of fish were feeding (pellets) from 10.6 to 16 mm when vision was available in light conditions (Figure 4). From 22.5 mm onwards, more fish were feeding when vision and mechanorception were available than for fish with only chemoreception (and inner ear mechanorception) (Figure 4).

4. **DISCUSSION**

Mulloway larvae appear to rely on vision for feeding in early development stages. A greater proportion of early larval mulloway were feeding in light conditions and consumed more food items than those fish fed in dark conditions. Once the mean larval length reached 5 mm, larvae began to consume food in dark conditions when mechanoreception was available. The role of vision became important again when fish were offered inert pellets with a greater proportion of fish feeding and a larger number of food items consumed in light conditions than in dark conditions. However, once the mean length of fish reached 22.5 mm, fish were again able to feed in the dark when mechanoreception was available.

The role of mechanoreception in marine fish larvae feeding has been well documented and studies of marine fish larvae have found that superficial neuromasts are capable of detecting disturbances in the surrounding water column. However, once neuromasts become enclosed in canals they become more receptive to water disturbances created by prey items and fish can develop the ability to detect food in dark conditions (Cobcroft & Pankhurst, 2003; Pankhurst, 2008). The lateral line canals, which contain neuromasts, also contain a gelatinous liquid that surrounds the receptive cells (Jørgensen, 1989). Disturbances created in the water column put varying amounts of pressure on the liquid within these canals, and a change in pressure consequently displaces the receptor cell causing a stimulus response (Denton & Gray, 1989).

The disturbances created in the water column by the movement of live prey items is likely to stimulate these receptive cells in mulloway larvae and may even enable feeding before the neuromasts are embedded in the canals of the lateral line. It does not however explain the increasing ability of more advanced mulloway to be able to feed in dark conditions when offered an inert pellet diet. It is possible that a feeding response is initiated by the water disturbances caused when pellets are initially offered, and then by the resulting movement of other fish within the experiment vessel, which has been suggested to stimulate feeding in fish in other studies.

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FIGURE 1

Number of (live) food items (mean \pm SEM) consumed by fish in 1 h. Fish were treated with streptomycin sulphate (SS) or salt water (SW) and fed in the dark or light. Different letters within a sample period indicate significant differences.



FIGURE 2

Number of fish (mean \pm SEM) feeding on live food in 1 h. Fish were treated with streptomycin sulphate (SS) or salt water (SW) and fed in the dark or light. Different letters within a sample period indicate significant differences.



FIGURE 3

Number of (pellet) food items (mean \pm SEM) consumed by fish in 1 h. Fish were treated with streptomycin sulphate (SS) or salt water (SW) and fed in the dark or light. Different letters within a sample period indicate significant differences.



FIGURE 4

Number of fish (mean \pm SEM) feeding on pellets in 1 h. Fish were treated with streptomycin sulphate (SS) or salt water (SW) and fed in the dark or light. Different letters within a sample period indicate significant differences.

4.6 The effect of temperature and salinity on the growth, feeding efficiency and survival of juvenile mulloway, *Argyrosomus japonicus*: An honours thesis submitted by Clint Becker (University of Newcastle)

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ABSTRACT

Mulloway, Argyrosomus japonicus, is a species identified as having good aquaculture potential. However as with many new aquaculture species, mulloway production is currently restricted by high production costs. In order to reduce these costs, improved production efficiency in hatcheries needs to be achieved through the optimization of such rearing conditions as water temperature and salinity. However, with little actual production and limited research to date, there is no information on the optimal growing conditions for juvenile mulloway. It would therefore seem that the establishment of such conditions required for optimal growth and feeding efficiency will play a key role in the development of future aquaculture efforts of this species. This study examined the effects of temperature and salinity on the performance of juvenile mulloway, held in 100-l experimental tanks at Port Stephens Fisheries Institute (PSFI). In the first experiment, performance of juvenile mulloway, each of approximately 9.4g in size, was assessed at four constant temperature treatments (17, 21, 26, and 31°C) over 28 days. Feed consumption had a positive linear relationship with temperature and significantly differed among all treatment temperatures, while growth increased with temperature up to 26°C, and then declined at 31°C. The food conversion ratios (FCR's) were exceptional at 21 and 26°C, with both being significantly lower than that at 31°C (0.60-0.62 compared to 0.84). The above demonstrated that the optimal temperature for growth and survival was 26°C, while the optimal temperature for FCR and condition is 21°C. It is therefore recommended that juvenile mulloway be reared in water temperatures ranging from 21-26°C.

In the second experiment, performance was assessed after transfer from 34% to four salinity treatments (5, 15, 25, and 34%) (water temperature $\approx 26^{\circ}$ C) for juvenile mulloway of approximately 26.4g in size, over a 14 day period. Juvenile mulloway were able to survive and grow at all salinity levels, however after 14 days there were differences in the performance of fish at these different salinities. Feed consumption was highest at 25‰, but was not significantly higher to that at 15‰. Growth showed similar trends as feed consumption, in that growth was improved at intermediate salinities. FCR was again best at intermediate salinities, and along with that at 34‰ was significantly lower than that of fish maintained at 5‰. The results indicate that growth and feeding efficiency of juvenile mulloway may be improved by rearing them at intermediate salinities, when water temperatures are optimal (i.e. 26° C).

In the third experiment, performance was assessed at combinations of two temperatures (21 and 26°C) and two salinities (5 and 34‰) in juvenile mulloway of approximately 15.2g in size, over a 14 day period. Feed consumption was not apparently affected by salinity but the consumption increased significantly as temperature increased. Growth was improved at 5‰, but not significantly so. Growth was significantly higher at 26°C at both 5 and 34‰ than those exposed to 21°C. FCR and survival were good for all treatment combinations and did not differ significantly. There were no interactive effects of temperature and salinity on any of the performance indicators.

Future investigation into ontogenetic changes in temperature and salinity optima may prove beneficial. Further research of the interactive effects of a larger combination of temperatures and salinities, including salinities within the intermediate range, may also be warranted.

1.1 History of aquaculture

Aquaculture is the production of aquatic organisms under either controlled or semi-controlled conditions (Mosig & Fallu, 2004; Stickney, 2005). Most of the publications on aquaculture or, more specifically fish culture, refer to the long history of this practice in Asia, ancient Egypt and central Europe (Pillay & Kutty, 2005). In some countries aquaculture has been practiced for thousands of years, however its origins appear to be embedded in China, possibly as long ago as 2000BC (Stickney, 2005). The first known written record describing aquaculture and its benefits was a book in Mandarin named the *Classic of Fish Culture* written by Fan Li around 500BC, in which he cited his fish ponds as the source of his wealth (Pillay and Kutty, 2005; Stickney, 2005). Hieroglyphics indicate that, throughout the Middle Kingdom (2052-1786BC), Egyptians also attempted intensive fish culture (Batis, 2006). Following on from the Egyptians, Romans also developed aquaculture practices, and along with growers from Greece and Japan, were the earliest oyster farmers, which is the first known form of aquaculture that has continued in some form or another up until the modern day (Batis, 2006; Pillay & Kutty, 2005).

All of the ancient forms of aquaculture differed significantly from much of the aquaculture practiced today. Probably the greatest difference is that aquaculture in the ancient times involved harvesting juvenile fish or shellfish and transferring them into an artificially created environment, where they were grown to a suitable size (Batis, 2006). Although in some instances this practice still continues today, for example tuna ranching, the majority of fish used in aquaculture today are sourced from hatcheries which spawn their own broodstock to produce juveniles. Aquaculture in its modern form was first established in 1733, when a German farmer successfully gathered fish eggs and sperm from trout during their spawning season. The sperm and eggs were mixed under favorable conditions and, after hatching, the fingerlings were transferred to tanks or ponds where they were cultivated (Batis, 2006). Initially this new form of aquaculture was restricted to freshwater fish, however during the 20th century new techniques were developed and saltwater species were successfully bred (Batis, 2006). Today aquaculture practices are relatively advanced, with growers having greater control over a fish's development.

1.2 Aquaculture in Australia

Aquaculture is currently the fastest growing primary industry in Australia, expanding at a rate of around 20% annually (CSIRO, 2006). The industry began in the late 1800's with the successful introduction of trout and the cultivation of the native Sydney rock oyster. The industry was centered on these two species until the first cultured pearl farm was established in north-western Australia in the 1950's (Dadswell, 2001).

During the 1980's a so called "new wave" of aquaculture development begun with the establishment of the Atlantic salmon industry in Tasmania and commercial cultivation of native freshwater fish, freshwater crayfish, prawns and pacific oysters (Dadswell, 2001).

Although aquaculture in Australia is still a relatively new industry, it has continued to grow and is now an important commodity in the Australian market place. At present there are over 40 species being commercially produced in Australia and, over the last decade, there has been a significant rise in aquaculture production from \$494 million in 1994/95 to \$732 million in 2003/04, which now accounts for almost one third of the total gross value production of the seafood industry (Love and Langenkamp, 2004). However, most of this production comes from high value species, such as tuna, salmon, edible oysters, pearl oysters and prawns, which contribute to about 90% of the gross value (Dadswell, 2001). During this same period the gross value of the Australian wild-caught fishery declined from \$1.83 billion to \$1.14 billion (Love & Langenkamp, 2004). In spite of the fact that aquaculture production in Australia has increased, it is still in its infancy when compared to the rest of the world. As increased aquaculture production occurs, suitable site availability especially for marine finfish farming may limit the expansion of the industry in some states of Australia, e.g. NSW and QLD (NSW DPI, 2006). Current research is attempting to solve this problem with investigations into the use of inland saline groundwater for the grow-out of marine fish. Results have been promising (Fielder et al., 2001) and, if this method is found to be adequate, a new industry could be developed relatively rapidly (NSW DPI, 2006).

1.3 Factors influencing growth in fish

In aquaculture, as in the natural environment, there are numerous factors, both biotic and abiotic, that can influence the growth of fish. However, the magnitude of these factors on growth can vary considerably between species and would therefore need to be investigated for each of those species being cultured. Water temperature and salinity are by far the two most important factors affecting the growth of fish (Kinne, 1963; Ojanguren et al., 2001; Fielder et al., 2005; Larsson & Berglund, 2005). Because of their importance in affecting fish growth, these two abiotic variables receive the most scientific attention when it comes to identifying the optimal growing conditions for any fish species.

Temperature can affect virtually all aspects of a fish's development, including those most important for growth, for example digestion, absorption, assimilation, metabolic expenditure and excretion (Ojanguran et al., 2001; Fielder et al., 2005). Numerous studies have demonstrated the ways in which growth can be significantly affected by water temperature and how the determination of optimal water temperatures can lead to maximization of growth and survival (Person-Le Ruyet et al., 2004; Fielder et al., 2005; Person-Le Ruyet et al., 2006).

Salinity, which is second only to temperature on the scale of factors affecting fish growth, can influence the amount of energy expenditure by fish in maintaining osmotic and ionic regulation (Neill, 1990; Foss et al., 2001). The amount of energy expended by fish to meet the metabolic costs of such regulation can be as high as 20-50% of a fish's total energy budget (Boeuf & Payan, 2001). Through simple manipulation of salinity regimes (usually a reduction from seawater), energy expended for osmoregulation may be reduced and instead be utilized for growth. This increased growth at lowered salinity regimes has been demonstrated by a number of fish species (Woo & Kelly, 1995; Partridge & Jenkins, 2002; Resley et al., 2005; Conides & Glamuzina, 2006).

Some of the other key factors affecting fish growth in aquaculture may include such physical environmental parameters as light intensity, photoperiod and stocking density (Hart et al., 1996) and also basic nutritional parameters such as feed composition, ration and feeding frequency. With any new emerging aquaculture species it is important to consider these factors as they will not only help to maximize growth but also help to reduce on-farm costs, which is probably one of the biggest factors restricting the growth of the aquaculture industry in Australia.

1.4 Mulloway

Numerous fish species are currently being farmed in Australia and several more species have been identified as having a good aquaculture potential. One of the species that appears to be a good candidate for aquaculture in Australia is the mulloway, *Argyrosomus japonicus*. Mulloway is a large species of sciaenid distributed in estuarine and marine waters of southern Australia, southern Africa, Pakistan, India, Hong Kong and Japan (Fielder & Bardsley, 1999). In recent years the interest in the culture of sciaenid fishes has grown significantly, with several species already being farmed on a commercial scale including red drum (*Sciaenops ocellatus*), black drum (*Pogonias cromis*), white seabass (*Atractoscion nobilis*), spotted seatrout (*Cynoscion nebulosus*) and orangemouth corvine (*Cynoscion xanthulus*) (Battaglene & Talbot, 1994), in countries such as South Africa and the USA.

Mulloway are common in Australia's southern waters (except Tasmania) with a distribution stretching from Bundaberg and the Burnett River in Queensland (24°45'S, 152°23'E) to North West Cape in Western Australia (21°56'S, 114°07'E) (PIRSA, 2001). They are a popular food fish fetching a high price and are highly prized by both commercial and recreational fishers. Although the biology and ecology of mulloway is not yet well understood, it is believed they spend most of their juvenile life in estuaries and near-shore waters, and migrate offshore as they reach maturity (Battaglene & Talbot, 1994; Fielder & Bardsley, 1999), suggesting they can tolerate a wide range of temperatures and salinities.

Mulloway have been targeted as an aquaculture species as they are widely distributed, have a good domestic market profile, are highly fecund and are euryhaline (Gray & McDonall, 1993; PIRSA, 2001). Mulloway also have a 'suitable' biology for aquaculture with fast growth and good food conversion ratios. Mulloway are a schooling fish and have the ability to adjust to captivity quite easily, which are also attributes that are important to the success of most cultured species (PIRSA, 2001).

As with many new aquaculture species, mulloway production is currently restricted by high production costs. In order to reduce these costs through improved production efficiency in hatcheries, it is important to optimize the conditions in which mulloway are reared (Ballagh et al., 2006). Such conditions may include the physical environment in which they are cultured (for example, temperature, salinity, light intensity and photoperiod) (Hart et al., 1996) and also the basic nutritional parameters such as feed composition, ration and feeding frequency. However, with little actual production and research to date, there is minimal information detailing optimal growing conditions for juvenile mulloway. It would therefore seem that the establishment of such conditions required for optimal growth will play a key role in the development of future aquaculture efforts of this species.

The purpose of my honours research was to identify the optimal temperature and salinity for growth and survival of juvenile mulloway and to also investigate any combined effects these two abiotic variables may pose. As little is known about the requirements of mulloway in aquaculture, the results of these experiments may help guide commercial growers in adjusting environmental parameters to exploit optimal temperature and salinity for growth of this species.

1.5 Thesis outline

- The general materials and methods, which describe those parts of each experiment which are common to all experiments, are provided in Section 2.
- Section 3 (experiment 1) examined the effects of four temperature (17, 21, 26 and 31°C) levels on the feed consumption, growth, feed conversion ratio, survival and condition, i.e. performance, of juvenile mulloway grown. The aim of this experiment was to identify the optimal temperature/s for growth and feeding efficiency of mulloway.
- Section 4 (experiment 2) examined the effects of four different salinity levels (5, 15, 25 and 34‰) on the performance of juvenile mulloway in order to identify the optimal salinity levels for growth and feeding efficiency for juvenile mulloway.
- The final results chapter (Section 5) describes the results from experiment 3, which examined the combined effects of two temperatures (21 and 26°C) and two salinity (5 and 34‰) levels on the performance of juvenile mulloway. The aim of this component of the thesis was to determine if there was any interaction between temperature and salinity on the performance of juvenile mulloway.
- Section 6 provides further interpretations and conclusions of the research, and recommendations for future studies.

2. GENERAL METHODS

2.1 Research facilities

All experiments were carried out in specially designed aquaculture facilities at the Port Stephens Fisheries Institute (PSFI). This facility was supplied with water, either from 35000L reservoirs of seawater (34‰) sourced from Tilligerry Creek (32°73'S, 152°05'E) in Taylors Beach, or from three smaller fibreglass tanks (1000 L) that contained reduced salinities (5‰, 15‰ and 25‰), which was a mixture of water sourced from Tilligerry Creek and treated ground water (Figure 2.1).

Prior to use, all water was treated with chlorine at a dosage of 200ppm for at least 12 hours before being neutralized with sodium thiosulphate. Soda ash (sodium carbonate) was also added to the reservoirs to maintain desired pH levels.

The 32 experimental and replacement tanks comprised of four rows, with each row containing eight tanks. Each of those rows was separately supplied water by a header tank. The four header tanks contained approximately 1,000 L, and were self filling from the reservoirs. Water was gravity fed from each of the header tanks to the respective row of tanks (Figure 2.2).





FIGURE 2.1

Reservoirs, (A) 35,000L and (B) 10,000L tanks, being utilized for the experiments at Port Stephens Fisheries Institute.

(B)

(A)



FIGURE 2.2

Arrangement of experimental and replacement tanks.

The experimental and replacement tanks were cylindrical in shape and had a cone shaped bottom, with a maximum capacity of 100L. Each of the experimental and replacement tanks had a water inflow pipe that carried water from the header tanks, a filter on the outflow, a standpipe, and a clear plastic lid. The filter and standpipe were covered with mesh so that fish could not escape or become trapped in the outflow or in the drainage pipe at the bottom of the tank when tanks were lowered. The dimensions of the tanks and the location of the apparatus just mentioned can be seen in Figure 2.3.



FIGURE 2.3

Experimental tank setup and dimensions

The aquaculture facility in which the experimental tanks were located was insulated and the temperature could be controlled with a refrigeration fan (Quadro 8-stage temperature control, Lovelocks, Australia). Unless otherwise stated all of the experimental and replacement tanks were equipped with 300W submersible glass heaters (Aqua One, Australia) (Figure 2.3) and the four header tanks were likewise equipped with 2KW submersible element heaters (Figure 2.4).



FIGURE 2.4

Header tank showing location of element heater.

All tanks received 12hrs light:12hrs darkness. Lights were located directly above each tank (Sylvania, dichroic 50W halogen, Germany) and the light intensity at the air-water interface was between 110-130lx (Mastersix Gossen, light meter, Germany).

All tanks were set-up as a flow through system (unless otherwise stated), with a flow rate of 300ml/minute entering the tanks. This resulted in an overall exchange rate of 430% within each tank on a daily basis, which was crucial in minimizing the accumulation of un-ionized ammonia levels. The flow rate was checked frequently to make sure all tanks had appropriate flows.

2.2 Experimental fish

The fish used for all experiments were juvenile mulloway (9.36g ± 0.05 g; mean \pm SEM, experiment 1; 26.43g ± 0.06 g mean \pm SEM, experiment 2; 15.22 g ± 0.05 g; mean \pm SEM, experiment 3) reared at the PSFI, and were the progeny of first generation broodstock. The mulloway were reared and held in 10,000L concrete tanks prior to the experiment and were fed a commercial feed manufactured by Skrettings Pty. Ltd., which comprised 50% crude protein, 20% crude lipid, 11% carbohydrates, 8% moisture, 11% ash, 1.8% total phosphorus and 1.1% available phosphorus.

2.3 Stocking procedure

Before the experimental and replacement tanks were stocked, oxy-tetracycline, which is an antibacterial, was added to each tank at a dosage of 100 mg/L. This dosage rate was intended to minimize any bacterial infection on fish due to stress incurred throughout the stocking process, and is current practice in aquaculture (Rigos et al. 2006).

The volume of the water in the concrete tank housing the fish to be used in the experiment was lowered to approximately 2500L. The water was then dosed with Aqui-S at a concentration of 10ppm, which anaesthetized the fish to reduce stress during handling. A random sample of 60 fish was then removed at the commencement of each experiment and the weight (0.1g) and standard length (mm) of each of those 60 fish was recorded so that an initial condition factor for the fish could be obtained. Fish were then removed by hand netting in lots of ten, weighed as a whole to the

nearest 0.1g and placed in the appropriate tanks. This process continued until all 32 tanks contained either 20 fish (experiments 1 and 3) or 10 fish (experiment 2) of approximately equal biomass. The experimental fish were left in the oxy-tetracycline for a 24hr period before the water was replaced. Following the flushing of oxy-tetracycline from each of the 32 tanks, the flow rates were adjusted to 300ml/minute and heaters activated to ensure the tank water was at the appropriate temperature. Salinities were adjusted where appropriate. Fish were left to acclimate in each tank for a three to four day period and fed once a day to satiation on the Skretting's commercial feed (see section 2.2.). No deaths occurred in any tanks during this time.

When each experiment commenced, the fish were fed to satiation twice daily, i.e. at 0900 and 1500 h, using the same feed and the amount was recorded. No uneaten feed was observed to accumulate in the bottom of the tanks.

The eighth tank in each row was utilized as a mortality replacement tank. Each mortality replacement tank was under the same conditions as all the other treatment tanks in that row. All dead fish were removed from tanks when they were noticed, and weighed. To maintain equal numbers of fish in each tank, replacement fish of similar size to the dead fish were caudal finclipped for identification, weighed, and placed into the tank from which the dead fish was removed.

2.4 *Water quality and tank maintenance*

Water quality (temperature, salinity, pH, and dissolved oxygen) was monitored on a daily basis throughout each of the three experiments using a Horiba U-10 water quality meter. On every second day, total ammonium (NH_4^+) was measured colourimetrically using a rapid test kit (E.merk, Model 1.08024, Germany). The experimental tanks were siphoned daily to remove any accumulated solids

2.5 Fish measurements

The total biomass (g) of the fish in each experimental tank was measured at 14 days after the experiment commenced and again at 28 days (experiment 1), when the experiment was concluded. The total biomass of each experimental tank at the 14 day sampling was measured by anaesthetizing the fish with 10ppm Aqui-S and weighing the fish in each tank as a whole in a pre-weighed bucket of water. Any mortality replacement fish were also individually weighed so that an adjusted biomass gain could be calculated. At the completion of the experiments, the standard length (mm) and wet weight (0.1g) were recorded for each individual fish.

2.6 *Performance indicators*

The following indices were calculated for each of the experimental tanks

- 1) **Feed Consumption (FC).** FC = mean total quantity of feed consumed throughout the experiment.
- 2) Adjusted Biomass Gain (ABG). ABG = (final total biomass + weight of dead fish) (initial biomass + weight of replacement fish) (Fielder and Bardsley, 1999).
- 3) **Feed Conversion Ratio** (FCR). FCR = Feed (dry weight)/ Weight gain (wet weight), where feed is the dry weight (g) of feed consumed per tank and weight gain is the adjusted biomass gain of each tank (Fielder and Bardsley, 1999).
- 4) **Survival (S).** Survival = 100^* (initial No. fish No. dead fish)/ (initial No. fish).
- 5) **Condition Factor (CF).** $CF = W/L^b * 1000$, where W is the weight (g) of the fish, L is the standard length (cm) of the fish and b is estimated by the length-weight relationship equation (W=aL^b) which is adjusted from data of the sampled individuals (Lima-Junoir *et al.*, 2002). CF was multiplied by 1000 to achieve a whole number.

2.7 *Statistical analyses*

The data for all experiments was firstly assessed for homogeneity of variance using Cochran's test and where necessary appropriate transformations were made. The performance indicators for each experiment were analyzed using either a one or two factor ANOVA. Where significant differences occurred Student-Newman-Keuls test was used to identify which means were different from one another. All statistical analyses were performed using *Statgraphics Plus* V4.1 (Manugistics Inc., Rockville, USA).

3. EXPERIMENT 1 – THE EFFECT OF TEMPERATURE ON GROWTH AND FEEDING EFFICIENCY OF JUVENILE MULLOWAY, ARGYROSOMUS JAPONICUS

3.1 Introduction

Temperature is the primary environmental factor controlling the growth rate of fish (Britz et al., 1997; Ojanguren et al., 2001; Van Ham et al., 2003; Fielder et al., 2005). The rates of all chemical reactions, including those that comprise metabolism, vary with temperature (Neill, 1990; Imsland et al. 1996). Fish are poikilotherms, and as such heat moves readily between the water and the fish, so in general the metabolic rate within the fish is set by the surrounding temperature of the water (Neill, 1990). Temperature can affect virtually all aspects of a fish's development, including those most important for growth, for example, digestion, absorption, assimilation, metabolic expenditure and excretion (Ojanguran et al., 2001; Fielder et al., 2005).

As a general rule the growth rate of fish has a parabolic relationship with temperature (Larsson & Berglund, 2005), i.e. growth rate increases with increasing temperature. However, when temperature becomes super-optimal, it can have a negative effect on growth (Van Ham et al., 2003). This negative effect on growth as water temperature reaches that super-optimal level, has been demonstrated for numerous species including brown trout (*Salmo trutta* L.), turbot (*Scopthalmus maximus*), pollack (*Pollachius pollachius*), European sea bass (*Dicentrarchus labrax*) and Australian snapper (*Pagrus auratus*) (Ojanguren et al., 2001; Person-Le Ruyet et al., 2004; Fielder et al., 2005; Person-Le Ruyet et al., 2006).

Negative impacts on growth, feed intake and utilisation can also occur at the lower limit of a fish's thermal tolerance (Borghetti & Canzi, 1993). If water temperatures become too low then fish activity will also decrease, their metabolism may slow significantly, and there is a concomitant reduction in feed intake. The exposure of fish to such temperatures that are at the upper or lower limits of its thermal tolerance can not only cause a reduction in growth, through deterioration of muscle tissue, but can also cause the fish to become stressed making them more susceptible to disease and bacterial infection and inadvertently lead to mortalities (Neill, 1990; Woo et al., 2002; Mosig & Fallu, 2004). Indeed several studies examining the effect of temperature on other fish species have encountered reductions in growth at both the upper and lower limits of thermal tolerance of those species and, in some situations high mortalities have occurred (Martinez-Palacios et al., 2002; Person-Le Ruyet et al., 2004; Fielder et al., 2005; Person-Le Ruyet et al., 2006).

The identification of the upper and lower limits of thermal tolerance for a fish species is essential information for commercial growers, as it enables the identification of the most suitable environments for the culture of their particular species. This information can also help determine the best time of year to rear juveniles, as fish are often subjected to extreme temperature conditions during pond culture in the different seasons throughout the year (Keembiyehetty & Wilson, 1998). Optimum water temperatures required for optimal growth are also species specific. Thus, some species of fish have large ranges of tolerance, e.g. migratory tuna (*Scombridae*), whereas tropical fish, which dwell in relatively warm waters, have a very narrow range of tolerance to temperature changes (Zimmerman, 2004).

In certain situations, even the same species can exhibit variations in their optimal temperature for growth (Van Ham et al., 2003). An example of this is the turbot, *Scophthalmus maximus*, where the same species from different geographical origin exhibits differences in its optimal temperature for growth. A study by Burel et al. (1996) determined the optimal temperature for growth of French turbot to be between 16 and 20°C, while Imsland et al. (2001), has reported that the optimal temperature for growth of Norwegian turbot to be between 19 and 24°C.

Having examined some of the effects of temperature on the growth of fish, determination of the optimal temperature for growth is not necessarily obvious. For example the temperature that produces the highest growth rates can also be the temperature that has the highest mortalities. A study by Martinez-Palacios et al. (2002) showed such a predicament, where they discovered that, although the temperature that produced the greatest growth in white fish, *Chirostoma estor estor*, was 28°C, survival was only 18% at this temperature. Further experimentation indicated that when both growth and survival were taken into account, the optimal temperature for growth was slightly lower, at 25°C. Furthermore, in determining the optimal temperature or temperature range for fish, decisions can not be made on growth alone, other, information such as food conversion ratios (FCR) and survivorship, also need to be incorporated into the selection process, as these are just as important to commercial growers, as achieving maximum growth.

With little actual production and research to date, there is little information detailing optimal growing conditions for juvenile mulloway, therefore it would seem that the establishment of the conditions required for optimal growth will play a key role in the development of future aquaculture efforts of this species.

The aim of this study was to determine the optimal temperature or temperature range for growth and feeding efficiency of juvenile mulloway as determined by an examination of performance indicators such as feed consumption (FC), adjusted biomass gain (ABG)(i.e. growth), food conversion ratio (FCR), condition factor (CF) and survival (S).

The hypothesis that was tested in this particular study was:

 H_A - there is a difference in feed consumption, growth, FCR, CF and survival of juvenile mulloway reared in different water temperatures (17°C, 21°C, 26°C and 31°C) for 28 days.

3.2 Methods and Materials

3.2.1 Experimental design

A single factor experiment was conducted, to determine the effects of temperature on the growth and feeding efficiency of juvenile mulloway.

Four temperatures, i.e. 17, 21, 26 and 31° C, were each investigated. Each treatment temperature was replicated seven times with each replicate tank containing 20 fish. The experiment was conducted over a 28 day period from the 10^{th} of April to the 8^{th} of May 2006.

3.2.2 Temperature control

All experimental and replacement tanks, except those selected to be run at 17°C, were equipped with 300W submersible glass heaters (Aqua One, Australia), so that the desired water temperatures could be maintained. The water temperature in the tanks without heaters was controlled via room temperature using the refrigeration fans.

The three header tanks for those rows with temperatures above 17°C also had 2KW submersible element heaters so that water entering the experimental tanks was at the temperature as in the experimental tanks.

3.2.3 Experimental procedure

Following the stocking of fish into the 32 tanks, the heaters were turned on to slowly bring the water temperature up to the desired levels. The fish were then left to acclimate to the increased temperatures for a period of three days before the commencement of the experiment.

Due to an outbreak of *Amyloodinium occellatum* in 3 of the experimental tanks in treatment 4 $(31^{\circ}C)$ after the first 14 day period, all tanks were given a 1-hour prophylactic formalin treatment (200ppm). A prophylactic treatment was then administered 2 more times, i.e. once a week until the completion of the experiment.

3.2.4 Statistical analyses

After 14 days (1st sampling) three experimental tanks being maintained at 31°C became infected with *Amyloodinium occellatum* and as a result all fish within those three tanks died. The data from these experimental tanks was not included in the final analyses. In order to maintain a balanced analysis, the data from four randomly chosen tanks, rather than the initial 7 tanks, within each of the other treatment temperatures was used.

The data for FC, ABG, FCR, CF and S were firstly assessed for homogeneity of variance using Cochran's test, and where necessary the data was appropriately transformed (ABG and FCR by log(x)) to meet assumptions of ANOVA (Underwood, 1997). The above five biotic variables were analysed using a single factor analysis of variance (ANOVA). Where significant differences were detected, the means were compared using a post-hoc Student–Newman-Keuls test to identify which means were significantly different from one another.

3.3 Results

3.3.1 Water quality

The salinity ranged between 33.57 and 34.36‰ in all treatments. Although both pH and dissolved oxygen tended to decrease, and total ammonium showed an increase with increasing temperature, such variations were not large. The measured water temperatures in each of the treatment tanks were very close to the predetermined temperatures for testing (Table 3.1).

TABLE 3.1

Mean \pm SE water quality (temperature, salinity, pH, dissolved, O2 and total ammonium) for each treatment over 28 days.

Treatment	Temperature (°C)	Salinity (‰)	рН	Dissolved O_2 (mg l ⁻¹)	Total Ammonium (mg l ⁻¹)
17°C	17.14 ± 0.34	33.57 ± 0.16	7.99 ± 0.02	8.33 ± 0.01	0.15 ± 0.03
21°C	21.02 ± 0.23	33.80 ± 0.18	7.88 ± 0.03	7.65 ± 0.09	0.13 ± 0.03
26°C	25.93 ± 0.06	34.16 ± 0.13	7.54 ± 0.03	6.51 ± 0.13	0.28 ± 0.04
31°C	31.21 ± 0.08	34.36 ± 0.22	7.39 ± 0.05	5.78 ± 0.13	0.35 ± 0.04

3.3.2 Performance indicators

After a 28 day period, ANOVA showed that temperature had a significant effect (P < 0.05) on feed consumption, ABG, FCR and condition of juvenile mulloway (Table 3.2). The mean amount of feed consumed by juvenile mulloway after 28 days was least at 17°C, i.e. 91.35g, and increased with increasing temperature, i.e. from 147.04g at 21°C, to 244.76g ± 11.89g at 31°C (Figure 3.1). SNK test ($\dot{\alpha} = 0.05$) showed that feed consumption was significantly different between pair-wise comparison of the four temperatures. Furthermore, the overall feed consumption had a positive linear relationship with temperature (Figure 3.1).

TABLE 3.2

Mean square values derived from ANOVA of the effects of temperature on feed consumption, adjusted biomass gain (ABG), feed conversion ratio (FCR), survival and condition of juvenile mulloway, *Argyrosomus japonicus*. df; degrees of freedom.

	df	Feed Consumption	ABG	FCR	Survival	Condition
Temperature	3	17480.5***	0.14***	0.02*	22.92	2.26***
Residual	12	242.52	0.01	0.005	27.08	0.17

***P< 0.001 *P< 0.05

Feed Consumption



FIGURE 3.1

Feed consumption of juvenile mulloway, *Argyrosomus japonicus*, grown at different temperatures (17, 21, 26 and 31°C) for 28 d. Data are means \pm standard errors (n = 4 tanks).

In the case of ABG (Figure 3.2), SNK showed that significantly higher values were recorded for fish in tanks at 21°C, 26°C and 31°C than at 17°C, but did not differ significantly between these higher temperatures at 28 days (Figure 3.2 (a)). Thus the adjusted biomass gain ranged between \approx 240 and 320g at 28 days for temperatures between 21 and 31°C, while it was less than 130g for 17°C. Similar trends were also evident 14 days after the commencement of the experiment, although the values were not as great (Figure 3.2 (A)).



FIGURE 3.2

Plots of (A) ABG and (B) feed conversion ratio of juvenile mulloway, *Argyrosomus japonicus* grown at different temperatures (17, 21, 26 and 31°C) for 14 and 28 d. Data are means \pm standard errors (n = 7 tanks for 14d, n = 4 tanks for 28d).

FCR (mean \pm SE) for each treatment after 28 days was 0.73 ± 0.07 at 17° C, 0.60 ± 0.01 at 21° C, 0.62 ± 0.01 at 26° C, and 0.84 ± 0.12 at 31° C (Figure 3.2 (b)). SNK test showed that FCR was significantly lower in the 21° C and 26° C treatments compared to 31° C, while that in the 17° C treatment was not significantly different to any other treatment.

The survival of juvenile mulloway after 28 days was 100% in tanks of fish at 17°C and 26°C. Although survival was highest within these two treatments, ANOVA showed that survival did not significantly differ overall (Table 3.2), with values of 97.5% and 95% being recorded for tanks at 21°C and 31°C, respectively.

At the commencement of the experiment, condition was approximately 19 (CF units) (Figure 3.3). At the conclusion of the experiment, the value reached ≈ 21 at 21°C and ranged between 19 and 19.5 for all other treatments (Figure 3.3). ANOVA and SNK confirmed that the condition at 21°C was significantly higher than that of the other temperatures. SNK also showed that the condition of fish grown at 17°C, 26°C and 31°C did not significantly differ from each other.



Condition

FIGURE 3.3

Condition of juvenile mulloway, *Argyrosomus japonicus*, grown at different temperatures (17, 21, 26 and 31° C) for 28 d. Data are means ± standard errors (n = 4 tanks).

3.4 Discussion

This is the first study of its kind that demonstrates the temperature dependence of growth, feeding efficiency and survival of juvenile mulloway grown in an aquaculture setting. The experiment clearly shows how temperature affects feed consumption, growth, FCR and survival of this species.

3.4.1 Comparisons of the different performance indicators

Feed consumption was at its lowest at 17° C, and thereafter increased significantly with each rise in temperature. This positive linear relationship between feed consumption and temperature has been described in numerous other studies examining the effects of temperature on food consumption of teleosts (Hidalgo et al., 1987; Burel et al., 1996; Person-Le Ruyet et al., 2004; Wuenschel et al., 2004; Larsson and Berglund, 2005). The strong linear relationship between feed intake and temperature ($R^2 = 0.992$) of this study is particularly similar to the study by Larsson and Berglund (2005), in which they determined a strongly significant linear relationship ($R^2 = 0.975$) between food intake and temperature for Arctic charr (*Salvelinus alpinus* L.). In contrast to this observed relationship, other studies have discovered different results between feed intake and temperature (Person-Le Ruyet et al., 2006; Sun et al., 2006). For example the study by Person-Le Ruyet et al.

(2006) found that feed consumption of juvenile pollack was not linearly related to temperature and that once feed intake reached its maximum at 18°C it dropped significantly at the next temperature increment of 21°C. This implies that juvenile mulloway are likely to be able to feed at higher temperatures.

Growth of juvenile mulloway, as determined by ABG, was not significantly different between temperatures of 21, 26 or 31°C. However, the growth was maximized and therefore determined to be at its optimum at 26°C. This is in line with results reported for other aquaculture species, for example sunshine bass (*Morone chrysops* \bigcirc X *Morone saxatilis* \bigcirc), white fish (*Chirostoma estor estor*), and European sea bass (*Dicentrarchus labrax*). Conversely it differs from such species as Pollack (*Pollachius pollachius*), brown trout (*Salmo trutta*) and Artic charr (*Salvelinus alpinus* L.) (Ojanguren et al., 2001; Larsson & Berglund, 2005; Person-Le Ruyet et al., 2006), however these differences may be a result of the different geographical origins of these species and the cooler waters they naturally inhabit.

Feed conversion was found to be optimal, and thus lowest at 21°C, but was not significantly different to that achieved by mulloway at 26°C. FCR at 17°C was exceptionally low for the first 14 days, after which the FCR increased significantly, suggesting some difficulties in adaption by mulloway to such low temperatures. Hidalgo et al. (1987), suggests that poorer FCR at low temperatures in general may be the result of lowered enzyme activity, which may explain the increase in FCR over the prolonged exposure at 17°C. The FCR's achieved at 21°C and 26°C were quite remarkable, and may be related to the ingestion of other food sources within the tank, as demonstrated in a study by Borghetti & Canzi (1993). They discovered through gut contents analysis that the fish in their experiment were also consuming algae and sediment other than that of the diet being fed. Although gut contents analysis was not performed in the present study, it is likely that the ingestion of alternate food sources, such as the bio-film within the tank, may have led to the very low FCR's achieved at 21 and 26°C. Future experiments utilizing gut contents analysis would confirm this. Compared to the other temperatures studied, the FCR at 31°C was poor and similarly higher FCR's at elevated temperature levels have been demonstrated in other studies (Keembiyehetty & Wilson, 1998).

Survival was not significantly different between the treatment temperatures but greatest survival was achieved at 17°C and 26°C, which was surprising considering 17°C would be close to the lower limit of thermal tolerance for mulloway (PIRSA, 2001). The condition of fish in all treatments improved on that of initial stocking. Condition of the fish was, however, significantly higher at 21°C then at any other temperature.

3.4.2 Experimental procedure and issues

Some problems were encountered throughout this experiment, primarily the outbreak of Amyloodinium ocellatum in tanks maintained at 31°C, 14 days into the experiment. Tanks at this higher temperature became infected with this parasitic dinoflagellate and, as a consequence, three tanks experienced total mortality of mulloway. This dinoflagellate often occurs in aquaculture systems, especially those utilizing intensive techniques (Johnson, 1990; Fielder & Bardsley, 1999). It is still not known how A. ocellatum entered the system; however tanks may have been compromised via either the influent water, by contamination due to daily maintenance, or through aerosol dispersal (Roberts-Thomson et al., 2006). This dinoflagellate apparently 'preferred' tanks at a temperature of 31°C, as fish in all other treatment temperatures showed no sign of infection. Although the optimal temperature range for reproduction of this ectoparasite has previously been identified as 18-30°C (Paperna, 1984), the time taken for the reproductive cycle to occur may be at its minimum at higher temperatures, explaining the observed outbreak at 31°C and not the other temperatures. It is possible that A. ocellatum was present in the other treatment temperatures, however because prophylactic formalin treatments were administered weekly to all tanks after the initial outbreak, A. ocellatum may have been unable to reproduce to a level that caused problems within these other treatment tanks.

One may be led to the conclusion that the depressed growth at 31° C, may have occurred due to the outbreak of *A. ocellatum*, however these assumptions would be unwarranted as the results of those tanks affected by this parasite were excluded from all analyses.

The arrangement of the facilities in which the experiment was conducted (see 2.1), meant that true replication of temperatures could not be achieved, instead due to the logistical constraints, pseudo-replication was incorporated. This resulted in rows containing the same temperature regimes for all tanks. It is highly unlikely that this impacted upon the results as the entire setup was receiving water from the same source.

3.4.3 Determination of optimal growth for mulloway in different water temperatures

Temperature has been shown to play an important role in governing the growth of many fish species through its effects on feeding and metabolism (Person-Le Ruyet et al., 2004). Growth was found to be markedly lower in cooler water (ABG was 60% lower at 17°C than at 26°C) and this drop in growth may have primarily resulted from the decrease in feed consumption (Person-Le Ruyet et al., 2004). Environmental stressors such as reduced water temperature have the ability to reduce metabolic rate within a fish, which can also be accompanied by a reduction in consumption, leading to no real enhancement of growth (Wuenschel et al., 2004). This may explain the significantly reduced feed consumption and near lack of growth achieved at 17°C in the present study. Conversely the increase in feed consumption at higher temperatures does not necessarily indicate increased growth (Wuenschel et al., 2004), as demonstrated by the reduction in growth at 31°C compared to that at 26°C. Increasing water temperature increases the demand for energy for maintenance and activity of the fish (Keembiyehetty & Wilson, 1998). Therefore the energy available for growth under such conditions is constrained by the physiological costs incurred at these temperatures.

Reductions in growth at temperatures above those identified as optimal, similar to that demonstrated in the present study have also been confirmed for numerous other species including brown trout (*Salmo trutta* L.), turbot (*Scopthalmus maximus*), Pollack (*Pollachius pollachius*), European sea bass (*Dicentrarchus labrax*) and Australian snapper (*Pagrus auratus*) (Ojanguren et al., 2001; Person-Le Ruyet et al., 2004; Fielder et al., 2005; Person-Le Ruyet et al., 2006).

The temperature responses of juvenile mulloway in terms of growth and feeding efficiency are similar to those of other studied teleosts (Keembiyehetty & Wilson, 1998; Tidwell et al., 1999; Martinez-Palacios et al., 2002; Cotton, et al., 2003; Pearson-Le Ruyet et al., 2004). For example, in European sea bass, *Dicentrarchus labrax*, temperature for maximum growth was 26°C, feed consumption increased with temperature and feed conversion was optimal at 22°C (Pearson-Le Ruyet et al., 2004).

The results of the present study suggest juvenile mulloway can tolerate higher temperatures (31°C), however given the reduction in growth, increased feed consumption, poor FCR and average condition of fish it appears 31°C is near the upper limit of temperature tolerance for this species. The apparent optimal temperature for growth and survival of juvenile mulloway is 26°C, while the optimal temperature for maximizing FCR and condition appears to be slightly lower at 21°C. These optima are similar to that reported for another sciaenid species, the red drum (*Sciaenops ocellatus*), which is said to prefer temperatures between 22°C and 25°C (Neill, 1990).

Although optimal temperatures for growth and feeding efficiency have been identified, these results may be limited to juvenile mulloway, as previous studies have shown optimal temperatures for growth can vary depending on age and size classes of the fish (Jonassen et al., 1999; Imsland et al., 2006). Further investigation into ontogenetic changes in optimal temperature may prove valuable.

The findings from this study may not be limited to aquaculture but may indeed be beneficial ecologically, as they may help to identify suitable areas for stock enhancement purposes. Fielder et

al. (1999) have identified mulloway as a possible candidate for enhancement of wild populations, as other similar sciaenid species, for example red drum and white seabass, have already been successfully restocked into rivers and lakes of other countries (NSW DPI, 2006). If stock enhancement is to be considered as an option for fisheries management large enough numbers of juveniles need to be produced and also once stocked, survival has to be high enough to have any beneficial effects. The results obtained in this study may help to maximize hatchery production of juveniles and also improve survival in the wild, through selection of areas with water temperatures similar to that identified as optimal for growth and survival.

3.4.4 Conclusion

This experiment demonstrates for the first time the temperature dependence of growth, feeding efficiency and survival of juvenile mulloway in an aquaculture setting. The temperature which resulted in the maximum response differed depending on the parameter measured: with the temperature at which feed consumption peaked being higher than that at which maximum growth was achieved, which similarly was higher than the temperature for optimal FCR. Taking into account all variables measured the optimal temperature range for growth and feeding efficiency of juvenile mulloway lies between 21 and 26°C. Due to a reduction in performance across all variables it is apparent that 31°C is close to the upper thermal tolerance limit for this species. The results obtained in this study may be restricted to certain size classes of juvenile mulloway or possibly even age classes, so further investigation into temperature/size relationships would prove beneficial.

This study has shown the capacity of mulloway to tolerate a large range of temperatures, reconfirming it as a species with good aquaculture potential. These findings have significant implications for the future aquaculture efforts of this species, primarily through the reduction of the high costs associated with current hatchery production of this species, and secondarily by making the grow-out of mulloway more profitable.

4. EXPERIMENT 2 – THE EFFECT OF SALINITY ON GROWTH AND FEEDING EFFICIENCY OF JUVENILE MULLOWAY, *ARGYROSOMUS JAPONICUS*

4.1 Introduction

After temperature, the most important environmental parameter governing the growth and survival of fish is salinity (Martinez-Palacois et al., 1990). In most fish species, salinity plays an important role in fish development and growth including, egg fertilisation and incubation, yolk sac resorption, early embryogenesis, swimbladder inflation and larval growth (Boeuf & Payan, 2001). In post-larval stages, salinity is also a key factor in controlling fish growth and feed consumption (Boeuf & Payan, 2001).

Salinity refers to the amount of solids dissolved and mostly ionised in water (Neill, 1990). The main contributors to the salinity of both seawater and the blood plasma of fish are Na⁺, Cl⁻, SO₄⁻, Mg²⁺, Ca²⁺ and K⁺, with the ratios and total concentrations of these ions differing considerably between seawater and that of the blood plasma of fish (Neill, 1990). The majority of fish regulate their plasma ions so that the internal osmotic pressure of their body fluids is about 10-15‰ (Brett, 1979; Foss et al., 2001). Marine fish species expend energy to meet the metabolic cost of osmotic and ionic regulation (Swanson, 1998; Foss et al., 2001), and the energetic cost to the fish in maintaining such regulation has been said to be 20 to > 50% of the total fish's energy budget (Boeuf and Payan, 2001), although recent studies suggest that it can be as low as 10% (Resley et al., 2005). Euryhaline fish, such as mulloway, are able to maintain near constant concentrations of ions in their blood plasma, despite the large variations in the environmental salinities in which they may live (Crocker et al., 1983; Neill, 1990), making such fish good osmoregulators. However, such osmoregulation requires metabolic input and the amount of this input must necessarily increase as the environmental salinity moves away from the optimum.

Because of the fact that salinity is an easily controlled variable and osmoregulation is an energydemanding process, it has been hypothesised that fish growth may be maximized in artificial settings by the selection of salinities that would decrease the energy expended in maintaining homeostasis (Tandler et al., 1995; Boeuf & Payan, 2001; Jarvis et al., 2001 Sampaio & Bianchini, 2002; Resley et al., 2005). A number of studies have examined the effects of salinity on growth of marine fish species (Martinez-Palacios et al., 1990; Tandler et al., 1995; Woo & Kelly, 1995; Partridge & Jenkins, 2002; Resley et al., 2005; Conides & Glamuzina, 2006), and showed that growth was increased or greater at intermediate or low salinities compared to those in seawater (~ 35‰). Similar results have also been obtained for mulloway and other sciaenid fishes (Fielder & Bardsley, 1999; Peterson et al., 1999). However in that former study on mulloway, the statistical power of the experiment was very low (0.22), and further investigation into the effects of salinity on growth of mulloway is therefore warranted.

Although a reduction in salinity leads to increased growth in some species of fish, it has been shown that for other species of fish, for example, bluefish (*Pomatomus saltatrix*), Nile tilapia (*Oreochromis niloticus*), spotted wolfish (*Anarhichas minor*) and European sea bass (*Dicentrarchus labrax*), that a reduction in salinity has no effect on the growth of these fish, with growth rates being either the same at all salinities tested or higher in those fish maintained at normal seawater (Dendrinos & Thorpe, 1985; Buckel et al., 1995; Ali et al., 1999; Foss et al., 2001). Furthermore, Swanson (1998) has shown that it is even possible for some species of fish to have improved growth at salinities higher than that of sea water. Swanson discovered that growth of milkfish (*Chanos chanos*) actually increased and was significantly higher in 55‰ compared to that of 35‰ and 15‰.

The optimal salinity regime for growth of fish thus appears diverse, as the various studies have shown how the growth of certain species responds differently to changes in salinity. However, there may be yet another difficulty associated in the determination of the optimal salinity for the growth of a certain fish species. Thus, as previously stated, the optimal salinity may change with life history stage (Crocker et al., 1983; Morgan & Iwama, 1998), requiring adjustment of the salinity regime to accommodate these changes.

One of the major shortcomings of most research into the effects of salinity on growth of fish is that they typically test two different salinities (Swanson, 1998), usually one that is close to fresh water and the other being normal seawater. However, if these studies are used to demonstrate that fish grow better at those lower or higher salinities, then it would be obviously meaningful to test intermediate salinities, from fresh to sea water, and even hyper-saline conditions to ascertain where growth is most rapid.

The aim of this study was to determine the optimal salinity or salinity range for the growth and feeding efficiency of juvenile mulloway as determined by feed consumption (FC), adjusted biomass gain (ABG), feed conversion ratio (FCR), condition factor (CF) and survival (S). The hypothesis that was tested in this particular study was:

 H_A - there is a difference in growth, FCR, feed consumption, condition and survival of juvenile mulloway reared in different salinities (5‰, 15‰, 25‰ and 34‰), and that fish grown at intermediate salinities will perform better.

4.2 Methods and Materials

4.2.1 Experimental design

A single factor experiment was conducted to determine the effects of salinity on the growth and feeding efficiency of juvenile mulloway.

Four salinities, i.e. 5‰, 15‰, 25‰ and 34‰, were each investigated. The treatment salinities were replicated seven times with each replicate containing 10 fish. The experiment was conducted for 14 days from the 19^{th} of June to the 3^{rd} of July 2006.

4.2.2 Salinity control

All tanks were set up as a static system, with each tank receiving a 70% water exchange twice daily. Water exchanges were conducted approximately 30 minutes after each feed.

The water utilized for the daily water exchanges was sourced from Tilligerry Creek and stored in four different reservoirs (3x10000L and 1x 35000L), with each containing one of the treatment salinities. All those salinities except 34% were achieved by diluting the estuarine water with treated ground water.

4.2.3 Experimental procedure

Following the stocking of fish into the 32 tanks, the salinities were lowered to the treatment levels of 25, 15 and 5‰ in 8, 16 and 24h respectively using treated groundwater. Once the salinities were at the desired level all heaters were activated, to increase water temperatures up to 26° C (see Chapter 3 for rationale). Fish were left to acclimate to the new conditions for a period of three days before the commencement of the experiment.

A one hour prophylactic formalin treatment (200 ppm) was administered to all tanks once a week to reduce the risk of infection by *Amyloodinium occellatum*, or the bacteria.

4.2.4 Statistical analysis

The data for FC, ABG, FCR, CF and S were assessed for homogeneity of variances using Cochran's test and wherever necessary, data was appropriately transformed (FCR by log(x)) to meet assumptions of ANOVA (Underwood, 1997). The above five variables were analyzed using a one-factor ANOVA to determine the effects of salinity on those variables. Where significant differences were detected (P < 0.05), the means were then compared using a post-hoc Student-Newman-Keuls test to identify which means were significantly different from one another.

4.3 Results

4.3.1 Water quality

Water quality (water temperature, pH, dissolved O_2 and total ammonium) was similar in all treatments for the duration of the experiment (Table 4.1). Mean water temperatures therefore ranged between 26.13 and 26.20°C, mean pH fell between 7.75 and 7.94, and mean total ammonium was 0.8 in all but the 15‰ treatment, where it was 0.87. The actual measured salinities were very close to those pre-selected for the experiment.

TABLE 4.1

Mean \pm SE water quality (temperature, salinity, pH, dissolved O_2 and total ammonium) for each treatment over 14 days.

Treatment	Temperature (°C)	Salinity (‰)	pН	Dissolved O_2 (mg l ⁻¹)	Total Ammonium $(mg l^{-1})$
5‰	26.13 ± 0.05	5.23 ± 0.06	7.94 ± 0.03	7.04 ± 0.19	0.80 ± 0.14
15‰	26.20 ± 0.06	14.93 ± 0.05	7.87 ± 0.02	6.77 ± 0.17	0.87 ± 0.08
25‰	26.18 ± 0.08	24.86 ± 0.07	7.80 ± 0.04	6.27 ± 0.16	0.80 ± 0.13
34‰	26.14 ± 0.06	33.94 ± 0.05	7.75 ± 0.04	5.90 ± 0.17	0.80 ± 0.13

4.3.2 Performance indicators

After a 14 day period, salinity was shown by ANOVA to have a significant effect (P < 0.05) on feed consumption, ABG and FCR of juvenile mulloway (Table 4.2). SNK test showed that feed consumption over this period was significantly higher in salinities of 15‰ and 25‰, with mean values of 87 and 87.5g, respectively (Figure 4.1 (A)). As for the feed consumption recorded for mulloway at 5 and 34‰, it was lower, i.e. \approx 84g, and did not differ significantly from each other.

TABLE 4.2

Mean square values derived from ANOVA of the effects of salinity on feed consumption adjusted biomass gain (ABG), feed conversion ratio (FCR), survival and condition of juvenile mulloway, *Argyrosomus japonicus*. df; degrees of freedom

	df	Feed Consumption	ABG	FCR	Survival	Condition
Salinity	3	25.16***	431.08*	0.01*	14.29	0.36
Residual	24	2.36	99.51	0.002	14.29	0.21

***P< 0.001 *P< 0.05



Feed Consumption



FIGURE 4.1

Plots of (A) feed consumption and (B) ABG of juvenile mulloway, *Argyrosomus japonicus*, grown at different salinities (5, 15, 25 and 34‰) for 14 d. Data are means \pm standard errors (n = 7 tanks).

Mean ABG for each treatment after 14 days was $73.81g \pm 5.41g$ at 5‰, $87.36g \pm 1.36g$ at 15‰, $92.34g \pm 4.18g$ at 25‰, and $83.41g \pm 2.87g$ at 34‰ (Figure 4.1 (b)). SNK test showed that ABG was significantly higher in salinities 15‰ and 25‰ than in those fish maintained at 5‰. ABG of fish grown in 15‰, 25‰ and 34‰ tanks were not significantly different from each other.

The mean FCR for all treatments ranged between 0.96 and 1.17 (Figure 4.2 (a)). SNK test showed that FCR was significantly lower in 15‰, 25‰ and 34‰ than fish grown in the 5‰ salinity, but did not differ significantly between these higher salinities.

ANOVA and SNK showed survival and condition were not significantly different between the four treatment salinities (Table 4.2). Survival within each of the treatment salinities was exceptional with 5‰, 25‰ and 34‰ having 100% survival, while 15‰ salinity had 97% survival.

At the commencement of the experiment condition was ≈ 17.3 (CF units) (refer to Section 2.6). At the conclusion of the experiment the values were very similar and ranged between 18 and 18.5. Surprisingly fish maintained at 5‰ were in slightly better condition, however there was considerable variation in the condition of fish between the replicates within this treatment salinity (Figure 4.2 (b)).

(A)



FIGURE 4.2

Plots of (A) FCR and (B) condition of juvenile mulloway, *Argyrosomus japonicus*, grown at different salinities (5, 15, 25 and 34‰) for 14 d. Data are means \pm standard errors (n = 7 tanks).

4.4 Discussion

4.5

This component of the study of juvenile mulloway, *Argyrosomus japonicus*, provided strong indications that the growth and feeding efficiency were influenced by salinity. Thus, significant differences between salinities were therefore detected in three of the five performance indicators, i.e. feed consumption, adjusted biomass gain (ABG), and feed conversion ratio (FCR).

4.4.1 Comparisons of the different performance indicators

Feed consumption of mulloway was significantly greatest at 15 and 25‰, similar to the results of Fielder & Bardsley (1999). At salinities of both 5 and 34‰, the total feed consumption was significantly less, implying that mulloway feed less at these 'suboptimal' salinities. However although these differences were shown to be significant using ANOVA, the actual real difference was only slight, being \approx 84g at 5 and 34‰ and \approx 87g at 15 and 25‰.

The appetite depression at suboptimal salinities similar to that just shown for mulloway has been observed in many other euryhaline species including another sciaenid species, the red drum (*Sciaenops ocellatus*), mullet (*Mugil cephalus*), gilthead sea bream (*Sparus aurata*) and black bream (*Acanthopagrus butcheri*) (DeSilva & Perera, 1976; Crocker et al., 1981; Dendrinos & Thorpe, 1985; Partridge & Jenkins, 2002). It is thought that such appetite reduction may be the result of a decrease in the secretion of hormones responsible for appetite induced by these low salinities (Le Bail & Boeuf, 1997).

The growth of mulloway, as measured by ABG, was significantly higher at 15 and 25‰ than at 5‰. The lower growth at 5‰ may be due to the increased osmoregulatory energy expenditure associated with the higher enzyme activity, required to maintain the plasma iono- and osmoregulatory balance at such low salinities (see Sampaio & Bianchini, 2002). Furthermore, growth in normal seawater (34‰) was not significantly different to any of the other salinities examined, emphasising the marine origin of this species.

Similar results in regards to higher growth at reduced or intermediate salinities compared to that of normal sea water have been demonstrated in numerous other fish species, including black bream (*Acanthopagrus butcheri*), black sea bass (*Centropristis striata*), cobia (*Rachycentron canadum*), European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), red drum (*Sciaenops ocellatus*), silver sea bream (*Sparus sarba*) and turbot (*Scophthalmus maximus*) (Crocker, 1981; Martinez-Palacios et al., 1990; Tandler et al., 1995; Woo & Kelly, 1995; Berlinsky et al., 2000; Imsland et al., 2001; Partridge &Jenkins, 2002; Cotton et al., 2003; Liaz-Carrion et al., 2005; Resley et al., 2005; Conides & Glamuzina, 2006). The results from the present study support the notion that fish growth may be maximized, by selecting intermediate salinities that help reduce the energy expended in maintaining homeostasis, thereby increasing the energy available for growth (Tandler et al., 1995; Boeuf & Payan, 2001; Jarvis et al., 2001 Sampaio & Bianchini, 2002; Resley et al., 2005).

While the growth results of the present study were similar to some of those found for other species of fish, they differed from another study of growth of mulloway in different salinities (Fielder & Bardsley, 1999). They reported higher growth of juvenile mulloway at 5‰ than that at 10, 20 or 35‰. However their study was based on fish of \approx 6g in weight, while the present study used fish of \approx 26g. Since previous studies have demonstrated that the optimal salinity for growth differ according to size of fish for some species it seems likely that such a difference in size may have contributed to the different results (Berlinsky et al., 2000; Cotton et al., 2003). Such a conclusion concurs with that for juvenile black sea bass (*Centropristis striata*) (Berlinsky et al., 2000; Cotton et al., 2000

The water temperatures used in both experiments also differed; Fielder & Bardsley (1999) used water temperatures of around 17°C, which in Experiment 1 of the current study have been

identified as suboptimal, while in the present study water temperatures were maintained at 26°C. Previous studies have shown that the optimal salinity for growth can be altered depending on temperature, for example Imsland et al. (2001) determined the best salinity for growth of juvenile turbot (*Scophthalmus maximus*) at 14°C was 34‰, while at 22°C, 15‰ salinity produced the greatest growth.

The food conversion ratio (FCR), of which lower values are the preferred result, did not differ significantly among the higher salinities, 15, 25 and 34‰, but were significantly lower than at 5‰. Improved FCR's at intermediate salinities in the present study are consistent with that of other studies (Imsland et al., 2001; Partridge & Jenkins, 2002; Conides & Glamuzina, 2006). For example, the greatest food conversion efficiency for gilthead sea bream was obtained between 18 and 28‰ (Conides & Glamuzina, 2006).

4.4.2 Determination of optimal growth of mulloway in different salinities

In the present study, comparison of feed consumption, ABG and FCR reveals that although feed consumption did not differ among the salinities, the ABG's were significantly greater at intermediate salinities and the FCR were relatively low at the three higher salinities. This collectively indicates that the optimal growth conditions for mulloway of 26g occur at both 15 and 25‰. The low FCR at 34‰, which was comparable to that attained at 15 and 25‰, was accompanied by lower growth, and therefore may be directly attributed to the significantly lower feed intake. These findings are in line with a study by Partridge & Jenkins (2002) who found that greater growth, feed consumption and most efficient FCR for black bream were achieved at 24‰ compared to that at 12, 36, 48 or 60‰.

Survival within all treatment salinities was excellent and was not significantly different between any of the salinities tested. The high survival across the large range of salinities reconfirms the recognition of mulloway as a 'good' osmoregulator and demonstrates its ability to tolerate large changes to environmental salinities, a feature that is important in most aquaculture species, especially those grown in pond culture.

Condition of fish was not significantly different between the treatments, and was very similar among all salinity regimes. Very few studies have taken into account condition factor when examining the effects of salinity on the growth of fish. This, along with the fact that length-weight relationships vary considerably between species, make it hard to formulate comparisons between different species. The only study found to include condition factor as a variable was that by Imsland et al. (2001), in which they found condition to be greater at intermediate salinities of 15 and 25‰, compared to that at 35‰.

Taking into consideration all of the variables measured in this particular study, the optimal salinity range for rearing juvenile mulloway appears to be 15-25‰. This is similar to another member of the sciaenid family the red drum (*Sciaenops ocellatus*), in which the optimum salinity for growth has been identified as 20‰ (Crocker et al., 1981). Further examination of salinity effects within this identified optimal range may help to narrow this range, and help pinpoint a definite optimum for growth and feeding efficiency of juvenile mulloway. These results may be limited to juvenile mulloway similar in size used in this study and may be of particular relevance to the hatchery production process and early growout stages of mulloway in commercial culture. Further investigation into ontogenetic changes in salinity optima may prove beneficial.

4.4.3 Conclusion

This study builds on previous work examining the effects of salinity on juvenile mulloway (Fielder & Bardsley, 1999). Although the findings of this current study differ in some respects from those studies, we all conclude that mulloway growth can be improved through a reduction in salinity of the water in which they are reared. The ability of mulloway to survive and grow under a large range of environmental conditions reconfirms its status as a good osmoregulator and a species that has great aquaculture potential.

With consideration of all variables measured it is recommended that juvenile mulloway be grown in water with a reduced salinity within the range identified as optimal for growth and feeding efficiency (15-25‰). The results of this study may be limited to juvenile mulloway and further investigation into ontogenetic changes in salinity optima will be beneficial to the industry, as it will help aquaculturalists determine the optimal salinity for the different size classes of fish grown in commercial enterprises.

The findings of this study have significant implications for the growout of mulloway in Australia as salinity is an easily controlled variable within those facilities. Adjustment of salinities can increase growth and feeding efficiency, which in turn will reduce the high costs associated with such growout efforts, therefore making aquaculture a more profitable and sustainable venture.

5. EXPERIMENT 3 – THE COMBINED EFFECTS OF TEMPERATURE AND SALINITY ON GROWTH AND FEEDING EFFICIENCY OF JUVENILE MULLOWAY, ARGYROSOMUS JAPONICUS.

5.1 Introduction

Although there are numerous studies on the individual effects of temperature and salinity on the growth of fish, almost none have investigated the combined effects of these two abiotic variables (but see Likongwe, 1996; Imsland et al., 2001; Fielder et al., 2005). Salinity has been reported to modify the effects of temperature on the growth of fish (Watanabe et al., 1993), and can also affect temperature preference of those fish (Stauffer et al., 1984). Furthermore water temperature may also influence the selection of salinity by individuals of fish species (Miller et al., 1983), due to the interactive effects of salinity and temperature on osmoregulation. Fielder et al. (2005) considers that, the two abiotic variables should always be considered together when trying to determine optimal conditions for fish tolerance and growth.

Of the few studies that have examined the combined effects of temperature and salinity on growth of fish there have been mixed results. Likongwe et al. (1996) observed significant effects of temperature, salinity and their interaction on growth of Nile tilapia (*Oreochromis niloticus*). They showed that at all tested salinities, growth increased with temperature, but, at all temperatures, an increase in salinity generally inhibited growth. These results were similar to that recorded by Imsland et al. (2001), who showed that the optimal temperature for growth of juvenile turbot (*Scophthalmus maximus*) varied with the salinity regime. Although these two studies did detect an interactive effect of temperature and salinity on growth, the same may not be true for all species (see Fielder et al., 2005). In this latter study, no interactive effects of temperature and salinity on growth were detected. However, the species that was the subject of this study was larval Australian snapper (*Pagrus auratus*), a stage which is likely to respond differently to abiotic variables than that of later or older individuals.

Because of the very limited research on interactive effects of temperature and salinity in aquaculture, it is difficult to assess the ways in which combinations of these variables will affect fish growth. However this is an area that warrants further attention, as water temperature and salinity often fluctuate within commercial aquaculture setups, particularly for those ventures utilising ponds for grow-out of their fish. If one can determine the optimal salinity for the different water temperatures that are encountered over the year, then appropriate manipulation of the salinities will be likely to lead to an increased profit and lowering of on-farm costs.

Mulloway achieved optimal growth performance at 21 and 26°C and 15 and 25‰, as evident by the results in chapters 3 and 4. The aim of this part of the study was to investigate the combined effects of temperature (21°C and 26°C) and two contrasting salinities (5‰ and 34‰) on the growth and feeding efficiency of juvenile mulloway as determined by four performance indicators of feed consumption, ABG, FCR, and survival.

The hypothesis that was tested in this particular study was:

 $\rm H_A$ - there would be a difference in feed consumption, growth, FCR, and survival of juvenile mulloway reared in different combinations of water temperature and salinity as a result of interactions between those two factors.

5.2 Methods and Materials

5.2.1 Experimental design

A two-factor experiment was conducted to determine the interactive effects of temperature and salinity on the growth and feeding efficiency of juvenile mulloway.

Two salinities were investigated (5‰ and 34‰) and were replicated across two environmental temperatures chosen from experiment 1 (21°C and 26°C). Each treatment combination was replicated seven times with each replicate containing 20 fish. The experiment was initially meant to run for 28 days however due to unforeseen problems the experiment was only conducted for 14 days from the 22^{nd} of May to the 5th of June 2006.

5.2.2 Salinity control

The two rows of tanks that were receiving normal seawater (35‰ salinity) were being pumped water from the 35000L reservoirs, while the other two rows of tanks that were testing 5‰ salinity had water supplied from two 10000L tanks using a submersible pump. The water supplied to all tanks was sourced from Tilligerry Creek. For the lower salinity water (5‰) the estuarine water was diluted using treated ground water.

5.2.3 Experimental procedure

Following the stocking of fish into the 32 tanks, all heaters were turned on to slowly bring water temperatures to the desired levels. In the two rows of tanks which were testing for the effects of 5‰ salinity, the salinity was lowered to the appropriate level over a 24 hour period, using treated groundwater. Fish were left to acclimate to the different conditions for three days before the experiment commenced.

A one-hour prophylactic formalin treatment (200 ppm) was administered to all tanks once a week to reduce the any risk of infection by *Amyloodinium occellatum*. The total biomass (g) of the fish in each tank was measured after 14 days, after which the experiment concluded due to problems involving anesthetic, in which the fish in the lower salinity tanks were exposed to residual anesthetic for a prolonged period of time and became compromised.

5.2.4 Statistical analyses

One of the tanks in treatment 21°C/34‰ experienced abnormal mortality due to poor water quality, which resulted in bacterial infection. As a consequence this caused an outlier in the data for survival and FCR, which in turn resulted in heterogeneous variance. Because homogeneity of variance could not be restored through transformation of these data, they were excluded from all analyses and replaced with the means of the other replicates (dummied data) to maintain equal numbers of replicates during the analysis. The degrees of freedom were adjusted accordingly.

The data for ABG, FCR, S and feed consumption were firstly assessed for homogeneity of variance using Cochran's test. Cochran's test was non-significant for all of the variables. All four variables were analyzed using a two-factor ANOVA to determine the combined effects of temperature and salinity on those variables. Where significant differences were detected, the means were then compared using a post-hoc Student-Newman-Keuls test to identify which means were significantly different from one another.

5.3 Results

5.3.1 Water quality

One of the tanks in the treatment combination 21°C/34‰ experienced abnormal mortality due to poor water quality, which resulted in the fish suffering bacterial necrosis. All data from this tank was therefore excluded from final analyses.

Except for the tank suffering bacterial necrosis, water quality was high and similar across all treatment tanks for the duration of the experiment (Table 5.1). The measurements of temperature and salinity showed that they are essentially the same as those outlined and examined in this experiment. The mean pH values ranged between 7.70 ($26^{\circ}C/34\%_{0}$) and 7.94 ($21^{\circ}C/5\%_{0}$), while the mean dissolved oxygen's were high and ranged between 5.86 ($26^{\circ}C/34\%_{0}$) and 7.86 ($21^{\circ}C/5\%_{0}$). The mean total ammonium was ≈ 0.2 in both $21^{\circ}C$ treatments and ≈ 0.4 in both $26^{\circ}C$ treatments.

TABLE 5.1

Mean \pm SE water quality (temperature, salinity, pH, dissolved O_2 and total ammonium) for each treatment over 14 days.

Treatment	Temperature (°C)	Salinity (‰)	рН	Dissolved O_2 (mg l ⁻¹)	Total Ammonium (mg l ⁻¹)
21°C/5‰	21.13 ± 0.09	5.39 ± 0.05	7.94 ± 0.04	7.86 ± 0.09	0.21 ± 0.03
21°C/34‰	21.17 ± 0.09	34.21 ± 0.16	7.85 ± 0.03	6.27 ± 0.11	0.24 ± 0.03
26°C/5‰	25.95 ± 0.09	5.37 ± 0.04	7.79 ± 0.05	7.32 ± 0.09	0.41 ± 0.05
26°C/34‰	25.89 ± 0.08	34.31 ± 0.18	7.70 ± 0.05	5.86 ± 0.13	0.43 ± 0.06

5.3.2 Performance indicators

Two-way ANOVA found no interactive effects of salinity and temperature on any of the performance indicators measured. Only water temperature was shown by ANOVA (P < 0.001) to significantly affect feed consumption and ABG, with both of these performance indicators being higher at 26°C (Table 5.2; Figure 5.1).

TABLE 5.2

Mean square values derived from two-way ANOVA of the effects of combinations of two temperatures (21°C and 26°C) and two salinities (5‰ and 34‰) on feed consumption, adjusted biomass gain (ABG) and feed conversion ratio (FCR) of juvenile mulloway, *Argyrosomus japonicus*. (No interaction values were supplied as no significance was detected by the statistical software used).

	df	Feed Consumption	ABG	FCR
Temperature	1	6990.45***	15662.2***	0.001
Salinity	1	2.26	736.51	0.01
Residuals	24	6.75	552.86	0.009

***P< 0.001

(A)



FIGURE 5.1

Plots of (A) feed consumption (B) ABG and (C) feed conversion ratio of juvenile mulloway, *Argyrosomus japonicus*, grown in combinations of two temperatures (21 and 26°C) and two salinities (5 and 34‰) for 14 d. Data are means \pm standard errors (n = 7 tanks).
Survival within all combinations of temperature and salinity (21°C/5‰, 21°C/34‰, 26°C/5‰ and 26°C/34‰) was high (Table 5.3). Condition factor was unable to be measured in this particular experiment, due to the experiment concluding after the first sampling at 14 days, in which only the biomass of each tank was measured. The experiment was stopped after 14 days because fish in 5‰ salinity at both temperatures were accidentally exposed to residual anesthetic for a period of 18hrs after the sampling, resulting in high mortality within these tanks.

TABLE 5.3

Survival of juvenile mulloway, *Argyrosomus japonicus*, grown in combinations of two temperatures (21 and 26°C) and two salinities (5 and 34‰) for 14 d. Data are means \pm standard errors (n = 7 tanks).

Treatment	21°C/5‰	21°C/34‰ ^a	26°C/5‰	26°C/34‰
Survival (%)	99.3 ± 0.71	99.2 ± 0.70	100	99.3 ± 0.71

^a An outlier was removed and replaced with the mean of the other replicates (dummied data).

5.4 Discussion

The effects of temperature-salinity interactions have never before been examined for juvenile mulloway. This is the first study of its kind that quantifies the effects of such interactions on performance indicators, such as feed consumption, ABG, FCR and survival, of this species. The results of such a study are important to the overall understanding of the requirements of juvenile mulloway in an aquaculture setting.

5.4.1 Comparison of the different performance indicators

It was determined that there were no interactive effects of temperature and salinity on any of the performance indicators for juvenile mulloway. Temperature was found to significantly affect feed consumption and ABG, while salinity had no significant effects on any of the parameters measured.

Although feed consumption was not influenced by salinity or by temperature-salinity interactions, it was significantly affected by temperature. Feed consumption was significantly higher at 26° C, at both 5 and 34%, than those fish maintained at 21° C, and is most likely the result of the higher metabolic rate within the fish at this temperature (Neill, 1990). The increase in feed consumption as water temperature was increased, at each of the salinity levels, is consistent with a study by Imsland et al. (2001). They discovered that food consumption of juvenile turbot (*Scophthalmus maximus*) increased with increasing temperature, from 10-22°C, at all of the salinity levels examined (15, 25 and 33.5‰).

While feed consumption may have increased with increasing water temperature, the opposite was found for consumption at the different salinities, i.e. at both temperatures, feed consumption increased slightly as salinity declined. This decline in feed consumption at higher salinities has also been demonstrated in juvenile sea bream (*Sparus aurata*) and Atlantic cod (*Gadus morhua*) (Lambert et al., 1993; Conides et al., 1997). Conversely juvenile flounder (*Platichthys flesus*) and European sea bass (*Dicentrarchus labrax*) were found to have increased feed consumption with increasing salinity (Dendrinos & Thorpe, 1985; Gutt, 1985).

The slightly higher feed consumption of juvenile mulloway held at 5‰ than that at 34‰ are in line with another study on the effects of salinity on juvenile mulloway by Fielder & Bardsley (1999), in which they found feed consumption of juvenile mulloway exposed to different salinity levels to be higher at 5‰ than those fish grown at 34‰. However, like the present study these differences were not significant and statistical power of the experiment was determined to be very low.

Growth of juvenile mulloway followed similar trends as that for feed consumption, in that temperature was again the only factor to significantly affect growth. Growth was markedly higher in those fish grown at 26° C, at both 5 and 34%, than that of fish grown at the same salinities at 21° C. These results are consistent with findings of previous studies, for example Likongwe et al. (1996) determined that growth of juvenile Nile tilapia (*Oreochromis niloticus*) increased with increasing temperature at all salinities examined and was significantly higher at 28° C than those fish maintained at 24° C.

The higher growth achieved at 5‰ salinity at 21 and 26°C, compared to that attained at 34‰ at the same temperatures supports the growth results obtained by Fielder & Bardsley (1999), in which they too found growth to be higher in mulloway at 5‰ than that at 34‰ salinity. However the water temperature in that study was slightly lower than those of the present study.

FCR's for all treatment combinations were excellent, with the lowest being recorded for the 26°C-5‰ combination. The relatively low FCR's obtained in all treatment combinations may again be related to the ingestion of alternate food sources within the tanks (see section 3.4). The FCR of fish grown at 5‰ salinity was lower at both temperature levels compared to fish grown at 34‰. This is comparable to the results obtained by Fielder & Bardsley (1999), in which they also found FCR of juvenile mulloway to be lower at 5‰ than that of fish grown at 34‰. The FCR's in the present study were however, substantially lower and may be attributed to the optimal water temperatures used in this study to the somewhat suboptimal temperatures utilised by Fielder & Bardsley (1999). The ability of fish to assimilate feed efficiently is of significant importance to commercial growers as it maximizes the growth of fish per amount of feed fed. If conditions can be manipulated to maximize FCR then the high costs associated with feeding, which are the greatest operating expense in aquaculture enterprises (De Silva & Anderson, 1995), may be significantly reduced.

Survival of fish among all treatment combinations was extremely high, and was significantly higher compared to that of snapper (*Pagrus auratus*) in a study by Fielder et al. (2005). However they were examining larval snapper, which are at a different life history stage to that of the juvenile mulloway used in the current study and may be more susceptible to mortality than that of juveniles. Survival, although similar in all treatment combinations, was slightly higher at 5‰ at both temperature levels than that at 34‰. This differs from the study by Fielder & Bardsley (1999), in which they found equal survival between these two salinity levels.

Mulloway's ability to survive and perform well at low salinities is a feature shared by other sciaenid species, for example, the red drum (*Sciaenops ocellatus*) and orangemouth corvina (*Cynoscion xanthulus*) (Crocker et al., 1981; Prentice, 1985). This ability to perform well at low salinities may explain the distribution of juvenile mulloway in the Hawkesbury River as reported by Gray and McDonall (1993), who found that the greatest numbers of juveniles were caught in sites where salinities were greatly reduced, while few were caught in marine-dominated sites near the mouth of the estuary. However there may also be some underlying processes other than salinity levels, such as food availability and risk of predation, acting upon such site selections in the wild.

In the present study there were no interactive effects of temperature and salinity on any of the performance indicators and these findings are consistent with results obtained by Fielder et al. (2005), in which they determined that the interaction of these two abiotic factors had no affect on growth or survival of snapper (*Pagrus auratus*) larvae. Alternatively other studies have confirmed interactive effects of temperature and salinity on growth and feeding efficiency of such species as juvenile turbot (*Scophthalmus maximus*) and Nile tilapia (*Orechromis niloticus*) (Likongwe et al., 1996; Imsland et al., 2001). However, these studies examined a larger range of temperatures and salinities than that of the current study, in which the scope (i.e. only two temperatures and two salinities examined) was limited due to logistical constraints, and further experiments examining a larger array of salinity-temperature combinations, including intermediate salinities, may lead to the detection of interactive effects of these factors on the performance of juvenile mulloway.

The growth-enhancing effect of lowered salinity at both temperature levels may primarily be explained by the increased feed consumption and improved FCR's. However, it may also be attributed to an increase in available energy for growth, as 5‰ may be closer than 34‰, to the internal osmotic pressure of juvenile mulloway, therefore requiring less energy to maintain homeostasis. However, the isosmotic value for juvenile mulloway is not yet known and identification of this value may confirm this assumption. The greater growth at the higher temperature of 26°C is mainly the result of increased feed consumption, brought about by the greater metabolic rate within the fish. The results indicate that growth, feeding efficiency and survival of juvenile mulloway may be improved through a reduction in the salinity in which they are reared.

5.4.2 Conclusion

Overall this study demonstrates that growth, feeding efficiency and survival of juvenile mulloway may be improved by rearing mulloway in low salinity water at temperatures identified as optimal (see Chapter 3). The results obtained may help in explaining the natural distributions of juveniles in estuaries, however there may also be some underlying processes that act upon the selection of such sites.

There were no interactive effects of temperature and salinity on any of the performance indicators; however the combination of 26°C-5‰ produced higher feed consumption, growth, survival and lower FCR than any other temperature and salinity combination. Further investigation into interactions between temperature and salinity, and their effects on the performance of juvenile mulloway over a larger spectrum, i.e. to include intermediate salinities, is needed and may provide differing results.

6. CONCLUSION

Quantifying the effects of environmental parameters, such as water temperature and salinity, on the growth and feeding efficiency of fish is an important process that needs to be performed for any new emerging aquaculture species. Not only does it give growers insight into the best conditions for rearing their particular species but it also helps to reduce the high costs associated with the aquaculture production of such species.

This study is the first of its kind to truly quantify the effects of water temperature, salinity and their interaction, on the performance of juvenile mulloway, *Argyrosomus japonicus*. This study has shown the capacity of mulloway to grow and survive under a large range of environmental conditions, reconfirming its current status as a species with great aquaculture potential.

- Juvenile mulloway were able to grow and survive over a range of environmental temperatures; however the optimal temperature range, in which growth and feeding efficiency was maximized, was between 21 and 26°C.
- The ability of mulloway to perform across a range of environmental salinities supports its status as a 'good' osmoregulator. The optimal salinity range which resulted in greater growth and feeding efficiency was at intermediate salinities of 15 and 25‰.
- No interaction between the two temperatures and two salinities on any of the performance indicators examined, for juvenile mulloway, was evident. However growth and feeding efficiency can be improved by rearing mulloway in low salinities and optimal water temperatures. Further investigation into combinations of a larger range of temperatures and salinities is needed, and may provide a better insight into the interactive effects of these two abiotic factors on the performance of juvenile mulloway.

The results of this study will be of significant importance for the establishment of mulloway hatcheries, as the previous lack of knowledge has been currently restricting the growth of production of this species. It will help to maximize the production efficiency in such hatcheries while at the same time reduce the high costs associated with current production. The results will

also benefit current and future growers of mulloway, and will be of particular importance to those growers who can manipulate environmental conditions. However it will also help those growers who utilize setups, e.g. floating cage culture, which cannot easily manipulate such environmental conditions, to select appropriate sites and times of year to maximize the performance of their mulloway.

7. FUTURE RESEARCH

The experiments conducted in this study are just the beginning of a long line of experiments that need to be performed to identify growing conditions that maximize growth, feeding efficiency and survival of juvenile mulloway. With regards to temperature and salinity, further investigation into ontogenetic changes in the optima for these environmental factors will prove beneficial, especially to those who wish to grow mulloway of different size and age, depending on market requirements. Further study into the interactive effects of temperature and salinity are warranted, and the use of a larger range of combinations including intermediate salinities and even suboptimal temperatures may help to identify optimal salinity and temperatures fall bellow that identified as optimal.

Some other key factors affecting fish growth that need to be investigated for mulloway include the effects of physical parameters, i.e. light intensity, photoperiod and stocking density, and also nutritional parameters such as feed composition, ration and feeding frequency. Examination of such parameters will help to build on the results obtained through this study, and give a better insight into how conditions may be manipulated to maximise the performance of mulloway.

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4.7 Effect of salinity and feed type on growth and survival of juvenile yellowtail kingfish, *Seriola lalandi*

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ABSTRACT

A two-factor experiment was done to determine the effects of salinity (15, 22.5 or 30 ‰) and feed type (Ridley, Australian-made or Biomar Optimal starter, Denmark) on growth and feeding performance of juvenile yellowtail kingfish (YTK), cultured in tanks for 14 days. There was no effect of salinity on growth or FCR of YTK however fish fed Biomar pellets were 30% heavier and FCR was 20% better than fish fed Ridley diet at the end of the experiment. There was no interaction between salinity and feed type. Results of this experiment suggest that sites with access to variable salinity seawater including estuaries may be suitable for land-based nursery or growout operations for YTK. We also recommend Biomar Optimal Starter as an early-stage diet for juvenile YTK.

1. INTRODUCTION

Optimising growth and survival of fish is generally essential for viable intensive aquaculture (Tandler et al. 1989). Performance of fish is influenced by a combination of abiotic and biotic factors with two of the most important factors being salinity and feeds. Many species of fish are euryhaline however salinity can affect growth and survival by influencing the amount of energy needed for osmoregulation (Howell et al., 1998). As salinity is increased, excess ions, which are accumulated by drinking sea water to replace osmotic water loss, must be actively eliminated using energy to stabilise extracellular fluid concentrations (Alderdice, 1988). Therefore compared with fish held in salinities more isosmotic to body fluids, fish held in higher salinities need to divert more energy into metabolism rather than growth.

Diet and nutrition have profound influence on the growth and survival performance of fish which in turn can be affected by salinity. Salinity can affect the rate of food consumption, absorption efficiency, conversion efficiencies, specific growth rate and allocation of energy to metabolism and growth (Qui and Qin, 1995). Culture of a particular fish species may therefore require the matching of salinity-specific diets to optimise fish performance.

Yellowtail kingfish, YTK, (*Seriola lalandi*) is a circumglobal species and in Australia is found in all coastal states where it is targeted by commercial and recreational fisheries (Fisheries Research Institute, 1988; Poortenaar et al., 2001). Culture of YTK in Australia is a new industry (Fernandes and Tanner, 2008) which is currently based entirely in the Spencers Gulf in South Australia using floating seacages. Research on culture of YTK has been done on reproductive physiology (Poortenaar et al., 2001), health (Sharp et al., 2004; Tubbs et al., 2005; Lackenby et al., 2006), larval development and rearing (Carton, 2005; Cobcroft et al., 2004; Moran et al., 2007; Chen et al., 2006; Chen et al., 2007) and metabolic physiology (Moran et al., 2007; Pirozzi and Booth, 2009) however little is known of the environmental conditions, including salinity, for optimal growth and survival of juveniles.

YTK are a pelagic, marine piscivore (Moran et al., 2007) and are found in a range of environments including inshore, shallow coastal waters to deep ocean habitats and are occasionally observed in estuaries when water quality is high and resembles ocean water. Based on these preferred environments it could be inferred that YTK require high and stable salinities for optimal performance, but this is unknown.

The choice of site and decisions for operating successful YTK farms require information on salinity tolerance to help managers avoid costly and potentially unwise business decisions. If YTK are stenohaline, then successful culture of the species will likely be limited in Australia to a small number of offshore, marine sites suitable for seacage culture. In contrast, if YTK can grow and survive well in a range of salinities then opportunities for their culture may be expanded to include land-based pond and closed, recirculating tank culture

Broodstock YTK maintained in land-based recirculating tanks at the Port Stephens Fisheries Institute survived up to 5 days where salinity of influent water declined from 30-32 % to 17 % as a result of a heavy rainfall episode (personal observation). During this low salinity period the YTK fed well and appeared to behave similarly to that shown in high salinity environments; however the long term effects of exposure to low salinity were not determined as tank salinity was returned to 30+% as soon as the estuary recovered from the freshwater influence.

Fish hatchery and nursery facilities are invariably land-based due to the tank and pond systems necessary to rear larvae and juvenile fish. Many coastal sites in Australia are situated on estuaries which are subject to changing water quality, especially salinity. Therefore an understanding of the salinity tolerance of juvenile YTK and also the potential impact salinity might have on susceptibility of YTK to ectoparasites is required.

Little is known about nutritional requirement of cultured YTK and research is being done to construct bioenergetic models and estimate requirements for digestible protein and energy for fish of different sizes. A range of feed ingredients have been analysed and their digestibility coefficients for juvenile YTK determined (Booth et al., this report). These data will then be used by feed mills to develop diets specific for YTK. In the meantime, the YTK industry is reliant on generic marine fish diets for nursery and growout operations. Australian made and imported diets are available, however the performance of YTK fed different diets has not been compared.

Comparison of non-species specific, commercially-available juvenile diets is necessary to provide information for hatchery and farm managers to choose an optimal feed in terms of fish condition and growth in conjunction with economics including cost and availability of the feed and cost of fingerling production. Although increasing, the marine fish farming industry in Australia is small (approximately 6842 t in 2007-08, ABARE, 2009) and production of commercial pellet diets is restricted to two major feed mills, including Ridley Aquafeeds. Importantly, manufacture of high quality, small, starter pellets for weaning of larvae from live feeds and for feeding to early-stage juvenile fish is expensive. Specialist feedmill equipment is needed to manufacture the pellets and volume of the starter pellets required to supply the Australian industry is small compared with the volume of large pellet grower diets. Therefore the monetary return on investment for Australian mills to produce starter fish feeds is questionable although some goodwill may be returned to the company for providing a service to the Australian industry. In contrast, the demand for starter feeds for the European temperate marine finfish industry is large and starter feeds are readily available from feed mills including Biomar Industries.

Ridley Aquafeeds required justification to continue production of marine fish starter feeds rather than to concentrate their production efforts on grower diets and to act as agents and provide a starter diet to the Australian industry by importing Biomar from Denmark.

The aim of the experiment was to determine the interactive effect of salinity and feed type on growth and survival of juvenile YTK reared in tanks.

2. MATERIALS AND METHODS

The experiment was done at the NSW DPI Port Stephens Fisheries Institute in March 2009. The experiment was designed as a 3x2 factorial experiment to determine the effects of salinity (15.0,

22.5, 30.0‰) and feed type (Ridley, Australian-made, Biomar, imported from Denmark to Australia) on growth and survival of juvenile YTK in tanks.

2.1 Source of juvenile kingfish

Fertilised YTK eggs were obtained from wild-caught YTK held in environment-controlled tanks at PSFI. YTK were induced to spawn following phototherm manipulation.

Larval YTK were stocked into a 10,000L outdoor, concrete tank and cultured using greenwater techniques until metamorphosis. After 30 days the juvenile YTK were cultured in flow-through seawater system until being used in the experiment.

2.2 Tanks used, experiment salinity, dilution schedule, stocking and feeding

The experiment was done in 24, 100L tanks described by Fielder and Bardsley (1999). Salinity and feed treatment combinations were imposed to randomly selected tanks (n=4 tanks/treatment combination). The salinity in the control treatments was maintained at 30 %, whereas salinities in the other treatments of 22.5 and 15 % were slowly lowered by adding groundwater (0.6 %) at 0.27L/min. Each day, 75% of the tank volume was drained and replaced with new water of the appropriate salinity.

During the experiment, salinity (± 1 ‰ of target salinity), water temperature (22-24 °C), dissolved oxygen (>6.0 mg/L) and pH (8.0-8.2) were measured daily using an Horiba U-10 meter (Horiba Ltd, Kyoto, Japan). Total ammonia-nitrogen (<0.4 mg/L) was measured daily from one randomly selected tank in each treatment.

Juvenile YTK were anaesthetised with 10ppm Aqui-S from the stock culture tank, dried on blotting paper, weighed individually and 6 fish of similar size were placed into each experiment tank (mean weight 1.25±0.15g/fish).

Fish were fed to satiation daily by hand at 0900 and 1500 h with either Ridley Aqua-feed; native fish starter or Biomar Optimal Start (Table 1) pellets. Pellets were provided slowly and observation was made to ensure they were eaten by fish in each tank. Feeding was stopped when fish did not eat 2 to 3 offered pellets. Pellet size was initially 1mm diameter and was increased to 1.5 mm pellets as fish grew. The amount of feed fed to each tank was recorded daily. Moribund or dead fish were removed from the tank and weighed as soon as they were noticed. Dead fish were not replaced. The experiment was terminated after 14 days at which time fish were harvested and the percentage mean fish wet weight, mean adjusted biomass gain = [final total biomass + weight of dead fish] – initial total biomass, and food conversion ratio (FCR) = [weight of food fed/adjusted biomass gain] were calculated for each tank.

TABLE 1

Nutritional declaration of commercial feeds

	Ridley native fish starter	Biomar Optimal Start
Standard		
Crude Protein (%)	52	56
Crude Fat (%)	12	18
Nitrogen Free Extract (%)	7	15
Ash (%)	13	11
Gross Energy (MJ/Kg)	19.6	21.5*

* estimated value. Not furnished with the feed

3. **RESULTS**

Survival of YTK was high for all treatments and 13 fish died after jumping from tanks. Kingfish grew in all treatments and there was no significant effect of salinity on final fish wet weight or FCR (Tables 2 and 3). Wet weight and FCR were however significantly affected by feed type. YTK fed Biomar diet were 32% heavier than fish fed Ridley diet after 14 days. FCR of YTK fed the Biomar diet was also 20% lower than fish fed Ridley diet (Tables 1 and 2). There were no significant interactions between salinity and feed type on final mean fish weight or FCR.

TABLE 2

Final wet weight and FCR of juvenile yellowtail kingfish *Seriola lalandi* grown in different combinations of salinity and diet for 14 days.

Salinity (‰)	Feed type	Final Wet Weight (g)	FCR
15	Ridley	4.2 ± 0.4	1.0 ± 0.05
15	Biomar	6.1 ± 0.4	0.8 ± 0.05
22.5	Ridley	4.4 ± 0.4	1.0 ± 0.05
22.5	Biomar	6.0 ± 0.4	0.7 ± 0.05
30	Ridley	3.8 ± 0.4	1.1 ± 0.05
30	Biomar	6.2 ± 0.4	0.7 ± 0.05

Data are means \pm standard errors. n = 4 replicate tanks

TABLE 3

Summary of analysis of variance for effects of combinations of three salinity (15.0, 22.5 and 30.0‰) and two diets (Ridley and Biomar) on juvenile yellowtail kingfish *Seriola lalandi* for 14 days.

Performance Index	Salinity (‰)	Feed type	Salinity x Feed type interaction
Final mean wet weight	ns ^a	<i>P</i> <0.00001	ns
FCR	ns	<i>P</i> <0.00001	ns

^a ns = not significant (P>0.05).

4. **DISCUSSION**

Juvenile YTK were remarkably tolerant to salinity in the range of 15 to 30 ‰ and growth and FCR were not affected when fish were cultured in these salinities. Little data exists for *Seriola spp.* which demonstrates the effects of salinity on growth performance and feeding efficiency and this is the first reported study for *Seriola lalandii*. Although no comparative study was done, Benetti et al. (1995) reported that growth to commercial size (1.4 kg in 6 months, 2.8 kg in 10 months), survival (90%) and FCR (2.8:1) of the Mazatlan yellowtail (*S. mazatlana*) was achieved in shrimp ponds in Ecuador where the salinity ranged from 10-18 ‰. Similarly Hu and Ji (2003) reported the biological parameters under which *S. dumerili* were successfully cultured from larvae to commercial harvest size and included a salinity ranging from 14-33 ‰; however no period of exposure to the lower salinity was discussed. Therefore this supports our findings and suggests that at least some *Seriola spp*. are tolerant of lower salinities, more typical of estuaries, despite the species generally occupying pelagic marine environments.

These results have implications for selection of sites suitable for YTK culture. Given the performance of juvenile YTK in a wide range of salinities in tanks it is likely that in-shore or landbased tanks and pond systems may be suitable for their culture, provided other water quality parameters including temperature, pH and turbidity are satisfactory.

In addition to YTK growing well at low salinities, ectoparasitic problems may also be reduced. *Benedenia seriolae* is a skin fluke which can cause significant mortality and large economic losses in commercial sea cage cultured YTK operations. A recent study by Ernst et al. (2005) demonstrated that viability of *B. seroliae* eggs was significantly affected by salinity and no eggs hatched at salinities less than and including 15‰. Culturing YTK at salinities of ≤ 15 ‰ will therefore preclude infestation with *B. seriolae* and potentially other ectoparasites including *Amyloodinium ocellatum* (Fielder and Bardsley, 1999).

Growth performance and FCR of juvenile kingfish were significantly better when fed the imported Biomar diet compared with the Australian-produced diet. This may be explained by the fact that the Biomar diet has a higher energy density than the Ridley diet, largely due to 30% more crude fat and higher crude protein concentrations. However, analysis of the feeds is necessary before detailed discussion of the diet differences can be made. Nevertheless, our study clearly demonstrated that the imported Biomar diet is a superior diet than Ridley native starter for YTK juveniles in terms of significantly greater growth rates, lower FCR's and consequent reduced time that fish are required to spend in the nursery. This information is now being used by Ridley Aquafeeds to determine whether they should continue manufacture of starter fish diets or discontinue manufacture and import Biomar starter diets and resell to Australian YTK farmers.

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4.8 Effect of fish size and feeding frequency on growth, survival and feeding performance of yellowtail kingfish, *Seriola lalandi*.

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ABSTRACT

A two-factor experiment was done to determine the effects of fish size $(3.0 \pm 0.03 \text{ g}, \text{small}; \text{ or 96.0} \pm 1.0 \text{ g}, \text{ large})$ and feeding frequency (1, 2, 4 times/d or continual) between 0900 and 1500h on growth and feeding performance of juvenile yellowtail kingfish, cultured in tanks for 35 days. Growth and FCR of juvenile kingfish was significantly affected by initial fish size and feeding frequency but there was no interaction between treatments. The average specific growth rate of small kingfish ($6.7 \pm 0.3 \%/d$) was 2.4 times greater than that of large juvenile kingfish ($2.8 \pm 0.03 \%/d$) and FCR of small fish (range 0.96-1.0) was significantly lower than that of large kingfish (range 1.1-1.3). For small kingfish, growth was greatest when feed was delivered 4 times/d compared with 2 times/d and continual feeding which did not differ. After 35 d, small fish fed 4 times/d were 40% heavier than small fish fed 1 time/d. In contrast, feeding frequency had no effect on growth of large juvenile kingfish at any sampling time during the experiment. Our results have implications for minimising cost of YTK fingerling production by understanding the optimal feeding strategy and use of hatchery labour for juvenile YTK of varying sizes.

1. Introduction

Optimising the growth and survival of marine fish juveniles, while reducing their cost of production is important for development of viable hatcheries and growout industry. Typically, cost of production of juvenile fish in emerging, start-up industries decreases in time as techniques and understanding of optimal rearing methods improve. For example in Australia, snapper, *Pagrus auratus*, culture is a relatively new industry and cost of fingerlings is approximately \$0.90-1.20/fish, whereas culture of barramundi, *Lates calcarifer* is a mature industry (>30 years) and purchase cost of fingerlings is approximately \$0.25/fish (Tucker et al., 2006).

Feeding can represent major costs to the production of fish in terms of labour input, the effects on growth, food conversion and food wastage, water quality and quality of fish including size variation within a cohort (Candreva et al., 1996; Tucker et al., 2006; Booth et al., 2008). Early stage and advanced juvenile fish grow rapidly however their growth rates and feed consumption can change significantly with ontogeny (Fielder et al., 2002). Frequency of feeding can significantly affect the performance of juvenile cultured marine fish including Australian snapper (*Pagrus auratus*) (Tucker et al., 2006; Booth et al., 2008) and Nile Tilapia (Riche, 2000). Hence it is essential to correlate growth stages with optimal feeding strategies. Culture of yellowtail kingfish, *Seriola lalandi*, YTK, is an emerging industry in Australia (Fernandes and Tanner, 2008) which is currently based entirely in the Spencers Gulf in South Australia using floating seacages. Research on culture of YTK has been done on reproductive physiology (Poortenaar et al., 2001), health (Sharp et al., 2004; Tubbs et al., 2005; Lackenby et al., 2006), larval development and rearing (Carton, 2005; Cobcroft et al., 2004; Moran et al., 2007; Chen et al., 2006; Chen et al., 2007) and metabolic physiology (Moran et al., 2007; Pirozzi and Booth, 2009) however little is known of the optimal strategies for feeding juvenile fish.

The aim of the experiment was to determine the effect of fish size and feeding frequency on growth and feeding performance of juvenile YTK reared in tanks.

2. MATERIALS AND METHODS

The experiment was done at NSW DPI Port Stephens Fisheries Institute and was designed as a 2x4 factorial experiment to determine the effects of fish size (small and large) and feeding frequency (1, 2, 4 times/d or continuous) on growth, survival and feeding performance of juvenile YTK.

2.1 Source of juvenile YTK

Two batches of fertilised YTK eggs were obtained from wild-caught YTK with approximately three months separating the batches (batch 1 and batch 2) in environment-controlled tanks at PSFI. YTK were induced to spawn following phototherm manipulation (Fielder et al., 1999).

For each batch, larval YTK were stocked into a 10,000L outdoor, concrete tank and cultured using greenwater techniques until metamorphosis. After 30 days the juvenile YTK were transferred to seawater recirculation tank facilities and cultured until being used in the experiment. Batch 1 was ongrown to a mean fish wet weight of 96g and was fed a series of commercial Ridley-Aquafeed (Narangba, Australia) pellets and was adjusted according to fish size i.e. Ridley Native Fish Starter 2mm, 3mm short, 3mm long (52% CP,12% CF), Ridley Barra Floating 6mm (50% CP, 12% CF). Batch 2 was ongrown to a mean fish wet weight of 3.0g and fed commercial pellet, Ridley Native Fish Starter 2mm.

Malformations of cultured YTK are common (Cobcroft et al., 2004) and all fish used for the experiment were selected initially for normal conformation. Fish from Batches 1 and 2 were anaesthetised with 10 ppm Aqui-S, captured by hand and inspected on both sides. Only fish without obvious deformities of the mouth, gill operculae, backbone and caudal peduncle were returned to the main holding tank for further stocking to the experiment.

2.2 Tanks used, experiment treatments, stocking and feeding

The experiment was done in 24 x 1000-L experiment tanks run on a recirculation system consisting of mechanical and biological filtration. Fish size and feeding frequency treatment combinations were assigned to randomly selected tanks (n=3 tanks/treatment combination).

Experiment treatments were fish size and feeding frequency (1, 2 or 4 times/d and continuous). Juvenile YTK were anaesthetized with 10ppm Aqui-S in the stock culture tanks, dried on blotting paper, weighed individually and stocked to the treatment tanks. A total of 30 small fish (mean wet weight 3.0 ± 0.03 g; small) and 10 large fish (96.0 \pm 1.0 g; large), were stocked in each of three replicate tanks for each treatment combination.

After stocking, the fish were maintained for 5 days to acclimatize to the tank conditions. This also allowed time to determine approximate feeding levels for the continuous treatment for each fish size. All tanks were fed to satiation by hand twice/d and the amount of feed consumed was recorded. The initial feed rate (%biomass/d) was then calculated based on the average daily amount of food consumed for 5 days and the initial biomass of fish for each size of fish.

Once the experiment was started, fish were fed by hand to satiation at 0900 (1 feed/day), 0900 and 1500 (2 feed/day), 0900, 1100, 1300 and 1500 (4 feeds/day) or continually on a belt feeder (AGK, Australia) which operated between 0900 and 1500h each day. The fish were fed commercial Ridley-Aquafeed diets and pellet size was adjusted during the experiment according to fish size: small fish - Ridley Native Fish Starter 2mm, 3mm short, 3mm long (52% CP,12% CF); large fish - Ridley Barra Floating 6mm (50% CP, 12% CF). For the 1, 2, or 4 feeds/day treatments, pellets were provided slowly and observation was made to ensure they were eaten by fish in each tank. Feeding was stopped when fish did not eat 2 to 3 offered pellets. The amount of feed fed to each tank was recorded daily. If necessary, daily adjustment of the amount of feed provided to the continuously-fed treatment was made. This was done by evaluating the amount of feed remaining

in the treatment tanks. If all feed given from the belt-feeder was consumed, the amount of feed provided the following day was increased by 10% of feed given on that day. In tanks where excess pellets remained, they were siphoned from the tank, counted and their total weight estimated according to their known mean dry weight/pellet. If $\leq 10\%$ of the feed given that day was not consumed, then the feed supplied on the following day remained the same. If $\geq 10\%$ of the feed given that day was reduced by 10%.

Moribund or dead fish were removed from the tank and weighed as soon as they were noticed. Dead fish were not replaced. Total biomass of each tank was measured after 14, 25 and finally at 35 days. On each occasion, the fish in each tank were anaesthetized with 10ppm Aqui-S, dried on blotting paper and weighed to provide data to calculate the percentage mean fish wet weight, mean adjusted biomass gain = [final total biomass + weight of dead fish] – initial total biomass, mean specific growth rate = 100* (In final mean wet weight – In initial mean wet weight)/days and food conversion ratio (FCR) = [weight of food fed/adjusted biomass gain] for each tank.

During the experiment, salinity (range 31 to 33 ‰), water temperature ($22-24^{\circ}$ C), dissolved oxygen (>6.0 mg/L) and pH (8.0-8.2) were measured daily using an Horiba U-10 meter (Horiba Ltd, Kyoto, Japan). Total ammonia-nitrogen (<0.4 mg/L) was measured daily from one randomly selected tank in each treatment.

The fish were held under ambient lighting and the experiment was run for 35 days. Treatment effects were significant at P<0.05.

3. **RESULTS**

Growth and FCR of juvenile kingfish was significantly affected by initial fish size and feeding frequency but there was no interaction between treatments. The average specific growth rate of small kingfish was significantly higher than that of large juvenile kingfish and FCR of small fish (range 0.96-1.0) was significantly lower than that of large kingfish (range 1.1-1.3) (Table 1). For small kingfish, growth was greatest when feed was delivered 4 times/d (Figure1) compared with 2 times/d and continual feeding which did not differ. After 35 d, small fish fed 4 times/d were 40% heavier than small fish fed 1 time/d. In contrast, feeding frequency had no effect on growth of large juvenile kingfish (Figure 2) at any sampling time during the experiment.

TABLE 1

Initial mean wet weight, mean final wet weight, mean SGR and mean FCR for kingfish of two sizes fed at different feeding frequencies (1, 2, 4 times/d or continual) for 35 days.

Data are means \pm standard errors, n = 3 replicate tanks



FIGURE 1

Effect of feeding frequency (1, 2, 4 times/d or continual) of commercial diet on growth of small juvenile kingfish. Data are means \pm standard errors, n = 3 replicate tanks. Different superscripts are significant at P< 0.05 for each time period.



FIGURE 2

Effect of feeding frequency (1, 2, 4 times/d or continual) of commercial diet on growth of large juvenile kingfish. Data are means \pm standard errors, n = 3 replicate tanks. There were no significant differences (P>0.05) between treatments for each time period.

4. **DISCUSSION**

Our results indicate that optimal feeding strategies for juvenile kingfish change with ontogeny and have major implications for management including labour and operating costs of fish in nursery and growout systems. Labour and electricity can represent up to 50% of the operating costs for juvenile marine fish production (Candreva et al., 1996) and feeding in fish nurseries is often a labour-intensive activity. Therefore an understanding of the optimal frequency of feeding fish and the commensurate input of labour is vital to minimise cost of fingerling production as well as optimise growth and condition of fish. Our data clearly show that for small fish from approximately 3-40 g, four, evenly-spaced hand-feeds between 0900 and 1500 produced the largest growth. Interestingly continual trickle-feeding from a belt feeder did not result in optimal growth for small fish. YTK are extremely active and aggressive when feeding and the more competitive fish may have established a feeding hierarchy below the belt feeder thus excluding access to feed by the smaller less competitive fish. Hand feeding can potentially avoid bias toward a feeding hierarchy because the feed is usually distributed evenly across the tank surface. However, belt feeders could be used to reduce the need for labour to feed if it was possible to arrange the feed on the belt to provide the feed in large pulses rather than continual trickle into the tank.

Of particular importance, our findings demonstrate that relatively small (approximately 100 - 250 g) fish only require feeding 1 time/d to maximise growth in 1000-l tanks. Fish of this size generally would be cultured in seacages where access to feed fish by boat is expensive and can at times be difficult due to poor weather conditions. Consequently, an understanding of the need for a single feed/d will allow cost-effective management of cage operations. Further research is needed to determine if feeding frequency can be reduced e.g. 1 time/2 days, as fish become larger.

FCR for both small and large juvenile YTK fed in tanks were approximately 1:1, although the FCR of the small was slightly better (lower). This is likely due to a minor increase (2%) in the protein content of diet fed to the small YTK compared with that fed to the larger juvenile YTK. Nevertheless, our data demonstrate that FCR is highly acceptable when YTK are fed to satiation in tanks.

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4.9 A preliminary study on extensive culture of yellowtail kingfish, *Seriola lalandi*, larvae in fertilised ponds.

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1. Introduction

Most marine fish larvae species are cultured using indoor, intensive techniques. This method is expensive and requires dedicated, sophisticated facilities and skilled labour (Fielder & Allan, 2008a). Briefly, this entails culture of larvae at high densities (50+/l) in clearwater or greenwater tanks generally ranging in size from 2000-l to 10,000-l. Larvae are fed rotifers, *Brachionus plicatilis*, from first-feeding until they are approximately 4-5 mm total length at which time the diet is changed to brine shrimp, *Artemia salina*. Both rotifers and Artemia require nutritional enhancement prior to feeding to fish larvae. Finally, larvae are weaned from artemia onto artificial pellet diets.

Alternatively, many freshwater and a small number of marine finfish including barramundi (*Lates calcarifer*), red drum (*Sciaenops ocellatus*), Australian snapper (*Pagrus auratus*) and mulloway (*Argyrosomus japonicus*) have been reared successfully using extensive methods in fertilised ponds (Fielder et al., 1999; Fielder & Allan, 2008a). Extensive methods rely on production of natural zooplankton in ponds filled with seawater and then fertilised to promote production of successive phyto- and zooplankton populations. This technique calls for a lower degree of sophistication and labour inputs can be less skilled and cheaper than intensive methods. The quality of fish produced by extensive methods can also be much greater than those produced using intensive methods, especially fish growth and conformation, likely due to low stocking densities and access to natural zooplankton including copepods (Fielder & Allan, 2008a). However, the success of rearing marine fish larvae can be influenced by a number of factors including age or maturity of the fertilised pond water prior to stocking, age of larvae at stocking into ponds and variable environmental conditions during the culture period (Fielder & Allan, 2008a; Fielder & Allan, 2008b).

Culture of juvenile yellowtail kingfish (YTK) is increasing in Australia (Fernandes and Tanner, 2008) and to date has been done exclusively using intensive hatchery methods (Kolkovski, 2007; Cobcroft, 2004). Fingerling quality has been highly variable and large percentages of some batches have sustained physical deformities including mouth, jaw and backbone deformities (Cobcroft, 2004). This is probably due to larval culture in sub-optimal abiotic conditions (e.g. salinity, temperature) or inadequate larval nutrition or both. Extensive pond rearing may provide a more natural environment for YTK larvae and produce high quality fingerlings.

The aims of this study were to determine if extensive pond techniques were suitable for larval rearing of YTK.

2. MATERIALS AND METHODS

Two attempts were made to evaluate if first-feeding YTK larvae could be cultured in ponds from 2007-2008 at the Port Stephens Fisheries Institute. Experiment 1 was done in June 2007 and Experiment 2 was done in April 2008. Larvae rearing experiments were originally planned to commence in January/February 2007 however we had difficulties spawning our captive YTK broodstock at this time. The broodfish had not matured as anticipated in the land-based tanks, however spontaneous spawning occurred following temperature and photoperiod manipulation in the Autumn of 2007 thus allowing us to commence larval rearing including extensive pond rearing trials.

In each experiment, two, 500 m² plastic-lined ponds were 2/3 filled with 500 μ m filtered seawater (30 ‰, Expt 1; 26.5 ‰, Expt 2) and then completely filled with seawater from a production pond which had been fertilised with organic and inorganic fertilisers to promote phyto- and zooplankton production using techniques described by Fielder & Allan (2008b). Both ponds were housed within a polyhouse. Experiments were run for 21 days when ponds were drained completely through a fish harvest net to retain any surviving larvae.

Experiment 1 was done in June when maximum water temperature was 16°C while Experiment 2 was done in April 2008 when pond water temperature ranged from 22 to 27°C. In both experiments, approximately 25,000 larvae (5 dah) were harvested from a stock, intensive greenwater tank and placed evenly into two, 100-l tanks which were located next to each pond and were filled initially with seawater from the stock tank. These tanks were then used to acclimatise the larvae to the recipient pond environmental conditions. This was achieved by pumping water from the experiment ponds into each respective acclimation tank. After 6 h and 1 h for Experiments 1 and 2, respectively the water quality parameters in the acclimation tank were similar to the pond parameters. All larvae were then transferred by bucket from each acclimation tank into the experiment ponds. Ponds were managed according to standard PSFI practices to maintain phytoand zooplankton blooms for three weeks, at which time ponds were drained to catch any surviving juvenile YTK (Fielder & Allan, 2008b).

3. **RESULTS AND DISCUSSION**

3.1 Experiment 1

No YTK larvae were harvested in either experiment pond. Broodfish YTK were induced to spawn at 22°C in controlled environment tanks and then the larvae acclimatised to the recipient pond water temperature which was approximately 16° C in June. This low temperature is likely to be suboptimal for successful larval development as incubation of YTK at <18°C led to poor hatching success (Moran et al., 2007). It is therefore unlikely that rearing YTK larvae during winter in extensive ponds is suitable. A second experiment was therefore done when potentially more suitable rearing temperatures were available.

3.2 Experiment 2

No juvenile kingfish were collected in netting attempts during the 21 day culture period and none were collected at final pond harvest. Acclimation of 5 dah larvae before transfer from acclimation tanks to ponds was rapid (1 h) because their water quality parameters were similar including pH (<0.1 unit difference), DO (range 8.6 -14.2 ppm), temperature (<1°C difference) and salinity (<2 ‰ difference). The reasons for total mortality of the kingfish larvae are unknown however posthandling mortality of early stage larvae is possible and may explain why no larvae were sampled during the culture period despite using rigorous netting techniques which are successful with other fish species including snapper and mulloway. Alternatively, the pond environment may not be suitable for survival of kingfish larvae. The water quality parameters of outdoor ponds tend to be more variable than those of intensive hatchery tanks and kingfish larvae may be susceptible to a changing environment.

Our preliminary results suggest that extensive pond culture of early-stage YTK larvae is not a suitable production platform, however further investigation should be done to determine if stocking advanced YTK larvae into ponds is more appropriate for production of juvenile YTK. Fielder & Allan (2008a) demonstrated that high quality snapper larvae can be produced when 16 dah larvae were transferred from intensive, indoor tanks to outdoor, fertilised ponds. The same may also be the case for YTK larvae.

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5. BENEFITS AND ADOPTION

- The research strategy of Aquafin CRC was developed explicitly to deliver the essential technologies needed by the Australian finfish farming industry, as identified by CRC participants. The industry partners defined the major goals they believed a CRC could achieve, and clearly indicated the weight of effort which should be applied to each of these goals. These goals and weightings were first defined at a workshop of potential CRC participants in December 1999. Partners involved in the current project included Ridley Aquafeed Pty Ltd, Silver Beach Aquaculture and Anthony O'Donohue (trading as O'Donohue Sand and Gravel Pty Ltd). During the planning period for the project, the South Australian Marine Finfish Farmers Association and the Stehr Group of Companies (at that time involved in marine fish farming and tuna grow-out) contributed to research priorities and plans.
- The industry participants continued to refine their priorities during subsequent development. Anthony O'Donohue leased Silver Beach operations and assumed obligations to the CRC from Silver Beach. Industry participants and researchers met regularly (several times per year) to discuss results, implications of results and how they might be used, and refined plans for subsequent experiments.
- Production of temperate marine finfish (predominantly mulloway and yellowtail kingfish with minor contributions from other marine species) in Australia increased to 1,763 t worth \$14.3 million in 2005/06 (O'Sullivan et al., 2008). There was no reported production of mulloway or yellowtail kingfish prior to 2001/02 (O'Sullivan and Dobson, 2003). Production is primarily in South Australia. Estimates for production in 2006/07 and beyond, indicate a significant increase in both the quantity and value of production.
- The research reported here has contributed in several major ways to Australian marine fish aquaculture:
 - Information on ingredient digestibility and nutritional requirements for mulloway and yellowtail kingfish has helped nutritionists formulate diets for marine fish diets.
 - Bioenergetic models for mulloway and yellowtail kingfish will assist farmers to plan feeding strategies, accurately estimate feed requirements, predict nutrient outflows from their farming operations, model growth and production for marketing purposes and populate farm financial models. The bioenergetic models will assist feed manufacturers to formulate diets to meet requirements for digestible protein and digestible energy (for fish at different phases of the growth cycle).
 - One of the most significant benefits is the confidence among feed manufacturers to use alternative ingredients to fishmeal and fish oil in response to reductions in availability and/or increases in price. The progression towards least-cost formulation (as is industry practice for terrestrial monogastric animal feed formulation) relies on rigorous ingredient evaluation, accurate estimation of nutritional requirements and systematic validation of different formulations. The increase in the price of fishmeal has made substitution a higher priority for feed manufacturers and customers concerned with rapidly rising feed ingredient prices. Some farmers remain adamant that fishmeal based diets are essential to ensure market access for exports of Australian farmed marine fish and for improved performance during sub-optimal environmental (e.g. temperature) conditions. Data provided in this report allows the cost of that strategy to be calculated.

- Hatchery practices and nursery technology were refined providing a much clearer understanding of the importance of abiotic factors (particularly photoperiod and light intensity), larval feeding strategies and diets on the cost-effective production of mulloway and yellowtail kingfish fingerlings. Information on feeding behaviour and the development of sensory organs in mulloway provides hatchery managers with a physiological basis for new feeding strategies.
- Low-cost technology for extensive production of mulloway larvae in fertilised ponds has been developed.
- The development of successful research methods cannot be underestimated as a benefit of this project. Research methods for diet development, larval rearing and nursery production have all assisted in the design of new research to address similar problems for other species.
- Research capacity at an institutional (Industry and Investment NSW and Ridley Aquafeeds) and personal level has been expanded.
- The aquaculture industry will benefit from new PhD qualified researchers focusing on nutrition and larval rearing.
- Adoption of nutritional information has been made possible because of the involvement of Ridley Aquafeeds throughout the project. Mulloway and yellowtail kingfish farmed in South Australia are using Ridley diets. Information has also contributed to formulations for other species sold to Australian farmers.
- Hatchery techniques have been shared with other hatchery managers and technicians during Aquafin CRC workshops and conferences, Australasian Aquaculture conferences and during specific meetings of marine finfish hatchery managers and technicians. A new Seafood CRC/FRDC initiative to support a marine finfish hatchery network to run regular workshops, support technical exchanges, fund travel bursaries and develop training activities for technical staff will help ensure Aquafin CRC technology is adopted for the benefit of Australian industry.
- The regular research meetings between Industry and Investment NSW and industry participants contributed greatly to the timely exchange of information between researchers and industry and the increased understanding of major issues affecting each group. This partnership is a major benefit realised from the Aquafin CRC and will assist with maintaining a strong R&D base for the temperate marine finfish farming

6. FURTHER DEVELOPMENT

Despite the commercial investment in snapper farming at the commencement of the Aquafin CRC, snapper farming has not developed in Australia and, without exception, operators moved to faster growing species such as mulloway and yellowtail kingfish. This progression was recognised during the Aquafin CRC and nutritional research and research to improve production of fingerlings shifted from snapper to mulloway and yellowtail kingfish. The industry in South Australia has also reduced emphasis on mulloway with increased focus on yellowtail kingfish.

For both species, data produced during this project will be published in the scientific literature (much of it is already published or "in press") and it will also be packaged in a format suitable for feed manufacturers and farmers. The finalisation of bioenergetic models for both species will allow feed manufacturers to accurately formulate diets for different size fish. Although it is likely feed manufacturers will only manufacture a small number of different specification diets for any one species, because of the cost of changing formulations and the relatively small demand, as production increases, information provided from this project allows feed manufacturers to formulate additional diets. The availability of digestibility data for both species gives feed manufacturers confidence to change ingredients within diets while maintaining required digestible protein and digestible energy contents. This is particularly important given the very rapid increases in fishmeal prices and the global search for alternative ingredients. During the course of this project, the cost of fishmeal has increased by at least 100% and at times has been virtually unavailable in Australia because of reductions in production and massive increases in demand, particularly from China. This rapidly changing supply/demand relationship for fishmeal has major implications for marine fish farming, particularly in Australia, where low production reduces the ability to negotiate large volume purchases of fishmeal at globally attractive prices. This reinforces the priority for continued investment in fishmeal replacement research.

Looking further ahead, demand to replace fishmeal and fish oil will increase and the importance of ingredient substitution will become more pronounced. It is likely that additional research will be needed to fine tune our understanding of how different ingredients perform in high specification diets for marine species. The relatively small differences in performance of ingredients of different cultivars of the same species (for grain-based ingredients) and different types of rendered animal products will become more important. Continues research into digestibility and utilisation of these ingredients, conducted in conjunction with feed manufacturers, will be a priority.

In terms of nutritional requirements, the modelling conducted during this project allows feed manufacturers to confidently formulate diets for optimum performance during environmental conditions that also approach optimum. However, fish farmers have reported sub-optimal performance during adverse environmental conditions (e.g. low temperature) and have speculated that improved performance during these conditions might be possible using new, different diet formulations, possibly also using different ingredients. This is a future research priority. The imperative for future nutrition research is to ensure whole-farm economics is considered when modelling different nutritional and feeding strategies. A key aspect is to ensure research is conducted with large fish most relevant to farming operations.

This project has led to considerable improvements in hatchery technology for mulloway to the stage where fingerling production for this species is reliable and routine. Broodstock management (including controlled phototherm regimes) has allowed fingerling production at any time during the year. Systematic research has determined the optimum combinations of photoperiod and light intensity for different age (size) larvae and juveniles and established optimum feeding regimes for larvae and juvenile to reduce feed and feeding costs.

Looking ahead, the key problem with yellowtail kingfish hatchery production is the high level of malformations. New research within the Seafood CRC is underway and is urgently needed to reduce levels of malformations to below 10%. This research will need to concentrate on nutrition and feeding, additional abiotic and biotic factors as well as innovative methods for screening malformed fingerlings at early sizes.

As the number and size of temperate marine fish hatcheries in Australia increases, the need to effectively share technology also increases. There is a strong need for more effective communication among hatchery technicians and managers to share advances and continually reduce the real cost of fingerlings. A marine fish hatchery network with regular meetings has already been established.

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7. PLANNED OUTCOMES

1. Profitable, sustainable and increasing industry for temperature marine finfish aquaculture in Australia. Achieved.

When the research programs for the Aquafin CRC were being designed, the key temperate marine finfish species being farmed or considered for farming in Australia was snapper (New South Wales, South Australia, Western Australia and Queensland). Research into mulloway was also underway on a small scale in New South Wales and South Australia. In addition, research into larval rearing for stripey trumpeter as an alternative species for salmonid farmers in Tasmania was underway. During the course of the Aquafin CRC, farmers who were culturing snapper were disappointed with slow growth rates and keen to trial other species. Research success with breeding mulloway and later yellowtail kingfish, made production trials with these species possible and farmers have progressively moved from snapper to mulloway to yellowtail kingfish. Production is concentrated almost entirely in South Australia although there are small operations in New South Wales and new, large farms being developed in Western Australia. Production trends are illustrated by the Figures below (compiled from Austasia trade Directories from 2001/02 to 2008). Recent advice is that production of mulloway in South Australia is likely to decline as farms increasingly shift their production to yellowtail kingfish and focus on production of southern bluefin tuna. Production estimates for yellowtail kingfish for 2006/07 exceed 2,000 t.



Production estimates (tonnes)



Production estimates by value (\$AUD)

2. Viable hatcheries, culturing high-quality, low-cost temperate marine finfish (for aquaculture or stock enhancement e.g. mulloway). Achieved.

Commercial hatcheries for marine fish are operating in South Australia, Western Australia, New South Wales and Queensland. The largest commercial hatcheries are operated in South Australia. In addition to commercial hatcheries, government owned hatcheries are operated in Western Australia, South Australia, New South Wales and Queensland. Contracts to produce fingerlings for commercial operations have been negotiated at all government hatcheries on a regular or irregular basis. Techniques for hatchery production of temperate marine species such as snapper, mulloway, yellowtail kingfish and stripey trumpeter have improved considerably over the period of the Aquafin CRC, particularly as a result of the projects on those species and communication among scientists and technicians working in the hatcheries. Communication has been facilitated by the Aquafin CRC meetings and workshops. The improvement is evident in higher survival rates, more consistent production and lower real costs on a per fingerling basis.

3. Reliable information on nutritional requirements for temperate marine finfish. Achieved.

For mulloway and yellowtail kingfish, bioenergetic modelling has been used to calculate requirements for digestible energy and protein (as well as allowing calculation of nutrient content in fish effluent and feeding rations for maximum growth). Estimation of requirements using bioenergetic modelling was confirmed using empirical dose-response studies for mulloway, increasing confidence in reported requirements. All experiments were designed in consultation with industry partner (Ridley Aquafeeds Pty Ltd) and results were made available as soon analyses were completed.

4. Reliable information on digestibility of available protein and energy sources for temperate marine finfish. Achieved.

For mulloway and yellowtail kingfish, apparent digestibility coefficients for protein, energy and organic matter have been determined for practical ingredients used in commercial and experimental diets. This information allows feed manufacturers to substitute ingredients (e.g. fishmeal) with other ingredients while maintaining a similar digestible protein and digestible energy content. This provides feed manufacturers with the data to respond to changes in ingredient availability and price and helps reduce feed price rises for farmers. The determination of digestibility data also allowed research to determine nutritional requirements.

8. CONCLUSIONS

- Mulloway eggs begin to hatch 28 h post fertilisation at 22°C
- Yellowtail kingfish eggs begin to hatch approximately 40 h post fertilisation at 22°C
- Mulloway eggs should be disinfected with a CT value of 1 at 22°C and the same procedure was successful for yellowtail kingfish
- Mulloway eggs (and we assume yellowtail kingfish eggs) can be treated at any stage of development and therefore should be disinfected with ozone early in development to reduce negative impacts of nodavirus and other pathogens on embryonic development.
- In early mulloway larvae:
 - length was significantly improved (P < 0.05) in fish held in 12, 18 and 24 h of light compared with fish in 6 and 0 h of light.
 - Survival was significantly (P < 0.05) reduced in 0 and 6 h of light compared with fish in 12, 18 and 24 h of light.
 - Swim bladder inflation was significantly higher (P < 0.05) in 6 h of light; however, there were no significant differences between SB inflation of fish in 12 h of light compared with any other photoperiod.
- In advanced mulloway larvae:
 - No effects were observed in growth at the end of the experiment.
 - Survival was significantly greater in the 0L:24D photoperiod compared with all other photoperiods.
- It is recommended that mulloway larvae are kept in a 12L:12D photoperiod until SB inflation occurs followed by a 18L:6D photoperiod to promote growth.
- Mulloway larvae can be weaned directly from rotifers to a pellet microdiet without the use of *Artemia*; however, this significantly reduces growth. Fish fed *Artemia* in combination with pellets also weaned successfully and displayed better growth rates, therefore the weaning process may be achieved more cost effectively by reducing the amount of *Artemia* used.
- Mulloway larvae began consuming *Artemia* in preference to rotifers once the mean length of larvae was 5.2±0.5 mm TL.
- Mulloway larvae began consuming pellets in preference to *Artemia* once the mean length of larvae was 10.6±1.8 mm TL.
- The most efficient feeding interval and photoperiod for rearing juvenile mulloway, *Argyrosomus japonicus* were determined by testing feeding intervals of one feed every 1, 3, 6, 12 or 24 h using a factorial experimental design with photoperiods of 12L:12D, 18L:6D and 24L:0D.
 - No interactions were found for any variables measured.
 - The 12L:12D photoperiod produced significantly poorer survival than other photoperiods, however feeding interval did not influence survival.
 - The 24L:0D photoperiod led to significantly reduced growth rates, compared to that of 12L:12D and 18L:6D.
 - Fish fed once every 24 h had significantly reduced growth, however there was no difference in the growth of fish fed every 1, 3, 6 or 12 h.
 - Juvenile mulloway should be reared in an 18L:6D photoperiod to promote growth and survival. Fish do not need to be fed more than twice a day or once every 12 h.
- Mulloway larvae rely on vision for feeding in early development stages.
- Once larvae reach 5 mm, the role of mechanoreception becomes increasingly important.
- Mulloway used vision for feeding when they were initially fed pellets; however, the role of mechanoreception again became important once the mean length was 22.5 mm.
- Yellowtail kingfish broodfish were successfully captured from Port Stephens and Botany Bay and held in controlled-environment tanks. Fish can be held indefinitely at 16°C and then induced to spawn reliably using temperature manipulation.

- Yellowtail kingfish larvae were reared in intensive tanks using greenwater methods. Survival from stocking to harvest of weaned juveniles was usually low (1-3%) and deformities (mouth, opercula and tail) of juveniles was high (up to 70% of fish affected).
- Further research is needed to determine optimal intensive larval rearing methods to improve quality of the fingerlings.
- For juvenile yellowtail kingfish, there was no effect of salinity (15, 22.5 or 30 ‰) on growth or food conversion ratio (FCR).
- Juvenile yellowtail (1.25 g/fish) fed Biomar pellets were 30% heavier and FCR was 20% better than fish fed Ridley starter diet.
- The average specific growth rate of small yellowtail kingfish $(6.7 \pm 0.3 \%/d)$ was 2.4 times greater than that of large juvenile kingfish $(2.8 \pm 0.03 \%/d)$ and FCR of small fish (range 0.96-1.0) was significantly lower than that of large kingfish (range 1.1-1.3).
- Growth and FCR was affected by initial fish size and feeding frequency but there was no interaction. For small kingfish $(3.0 \pm 0.03 \text{ g})$, growth was greatest when feed was delivered 4 times/d. After 35 d, small fish fed 4 times/d were 40% heavier than small fish fed 1 time/d. In contrast, feeding frequency had no effect on growth of large $(96.0 \pm 1.0 \text{ g})$ juvenile kingfish at any sampling time during the experiment.
- Preliminary trials aimed at rearing yellowtail kingfish larvae (5 dah) using extensive rearing methods in fertilised ponds at PSFI were unsuccessful. Low temperatures (16°C) were too low and post-stocking mortality was suspected. Further research with older larvae is recommended.

9. APPENDICES

9.1 Intellectual Property

All information brought into this project or developed during the project is public domain.

9.2 Staff

- Dr Geoff Allan, Principal Investigator/Research Leader, Aquaculture and Port Stephens Fisheries Institute Director.
- Dr Mark Booth, Co-Investigator/Research Scientist, Fish Nutrition, Port Stephens Fisheries Institute.
- Dr Stewart Fielder, Co-Investigator/Research Scientist, Marine Fish Breeding, Port Stephens Fisheries Institute.
- Mr Ian Russell, Fisheries Technician, Port Stephens Fisheries Institute.
- Mr Luke Cheviot, Fish Hatchery Manager, Port Stephens Fisheries Institute.
- Mr Paul Beevers, Fisheries Technician, Port Stephens Fisheries Institute.
- Mr Luke Vandenberg, Fisheries Technician, Port Stephens Fisheries Institute.
- Mr Ben Doolan, Fisheries Technician, Port Stephens Fisheries Institute.
- Mr Igor Pirozzi, PhD Student, James Cook University.
- Ms Debra Ballagh, PhD Student, James Cook University.
- Clint Becker, Honours Student, University of Newcastle.



Research Fact Sheet

DRAFT

AQUAFIN CRC: FEED TECHNOLOGY FOR TEMPERATE FISH SPECIES

OT AL

introduced to you in

Objectives:

out diets.

1.

3.

subsequent Fact Sheets.

To reduce costs of

fingerling production.

effectiveness of grow-

To validate improved

feeds and feeding

commercial scale

practices on a

Experiments:

About 30 experiments are scheduled for completion

conducted at NSW DPI's

Port Stephens Pisheries

Centre, Taylors Beach.

Future Fact Sheets:

by May 2009. These will be

Research Fact Sheets in this

series will provide a brief

description of completed

experiments and results.

To improve the cost-

Research Partners: Ridley Aqua-Feed Aquafin CRC FRDC NSW DPI Clearwater Mulloway

> Species under Research:







Aquatin CRC



Australian Government **Fisheries Research and**





INSW DEPARTMENT OF PRIMARY INDUSTRIES



Why do this research?

This research follows on from the very successful project "Increasing the profitability of snapper farming by improving hatchery practices and diets". The research in this project was listed as a priority area for research by the Aquafin CRC Joint Management Advisory Committee, The Yellowtail **Kingfish Aquaculture** Strategic R&D Plan 2003-2008 and at the Aquafin CRC Snapper Workshop, 2003. It has been requested by marine fish farmers in NSW and SA and by the largest feed manufacturer in Australia - Ridley Agriproducts. Two PhD students from James Cook University were appointed to work on this project at Port Stephens Fisheries Centre (PSFC) and their research will be profiled in the next Fact Sheet.

Over \$3 million will be injected into this project over 4 years:

- \$977,905 from FRDC \$380,567 from industry
- partners \$1,851,489 from NSW DPI

Facilities & staff at PSFC:

Research facilities at PSFC are second to none and include spawning and larval rearing tanks, production units for algae, rotifers and brine shrimp and lined ponds for extensive larval rearing. Dr Geoff Allan is Principal Investigator on the project assisted by **Research Scientists Drs** Stewart Fielder and Mark Booth. The team of 7 technicians will be



Introducing ...

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Introducing... **Our industry partners**



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Aquafin CRC Feed Technology Final Report Vol. 1, Fielder et al.



Research Fact Sheet

TEMPERATE FISH SPECIES

Research Partners:

Ridley Aqua-Feed Aquafin CRC FRDC NSW DPI **Clearwater Mulloway**











The title of Debra Ballagh's project is "Improvement of intensive rearing of larval and juvenile mulloway (Argyrosomus japonicus) and yellowtail kingfish (Seriola lalandi lalandi)." The major aims of her research are:

- 1. To determine the best combination of live feeds/inert feeds for larval mulloway and yellowtail kingfish.
- To determine the best weaning and feeding strategies for juvenile mulloway and yellowtail kingfish.

Why do this research?

A reliable supply of cheap, high quality healthy fingerlings is essential for sustainable mulloway and yellowtail kingfish farming. Fingerling costs for mulloway and kingfish are currently estimated at \$0.60-\$2.00/fingerling. This cost may represent approximately 10% or more of the total production cost. To improve profitability, there is a need to reduce the cost and improve the quality and vigour (optimize growth) of fingerlings and to develop cost-effective high-performance diets and feeding systems for both hatchery and grow-out. This project will improve hatchery methods and potentially replace live





PhD Students Debra Ballagh and Igor Pirozzi

feeds, including artemia which are expensive and can have unreliable supply, with alternative live feeds or artificial feeds. The project will also determine feeding strategies (frequency of feeding, density of fish, artificial day-length) to optimise fingerling survival, growth, quality and population size variation. Cost of production will be reduced by increasing the

turnover rate of fingerlings from the hatchery-nursery systems and thus allow more hatchery runs to be done each year.

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The title of Igor Pirozzi's project is "Comparison of protein and energy requirements of mulloway and kingfish using a factorial modeling approach". The major aims of his research are:

1. To construct and test factorial models based on the protein and energy requirements of rapidly growth mulloway and kingfish. 2 To compare differences in requirements for protein and energy for yellowtail kingfish and mulloway.





Why do this research?

Lack of nutritional information on mulloway and kingfish in Australia is limiting the development of their aquaculture potential. At the moment farmers rely on commercial diets formulated for other species. These diets produce results, but it is unknown if they are nutritionally adequate, especially for rapidly growing fish. Generally, the most important initial nutritional information for diet development is the determination of specific digestibility coefficients for potential feedstuffs. Secondly, the gross nutrient requirements for a fish species needs to be established. Because of the implications for the cost of diets, the requirements for protein and energy are usually determined before requirements for other nutrients. In addition, farmers demand feeds that are highly efficient in terms of feed utilization and that produce lower nutrient outputs from their farms.

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Debra and Igor are conducting their research at Port Stephens Pisheries Centre. They are on track to finish their PhD's this year and results of individual experiments will be presented in subsequent Research Fact Sheets.
Final Report



AQUAFIN CRC PROJECT 1B5: FEED TECHNOLOGY FOR TEMPERATE FISH SPECIES VOLUME 2: DIET DEVELOPMENT

Mark A. Booth, Igor Pirozzi, Geoff L. Allan & D. Stewart Fielder





July 2010

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AQUAFIN CRC PROJECT 1B5: FEED TECHNOLOGY FOR TEMPERATE FISH SPECIES VOLUME 2: DIET DEVELOPMENT

Mark A. Booth, Igor Pirozzi, Geoff L. Allan & D. Stewart Fielder

July 2010

FRDC Project No. 2004/220









Australian Government

Fisheries Research and Development Corporation



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NON-TECHNICAL SUMMARY

2004/220	Aquafin CRC – Feed Technology Temperature Fish Species: Volume 2: Diet Development					
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OVERALL OBJECTIVES:

- 1. To reduce costs of fingerling production.
- 2. To improve the cost-effectiveness of grow-out diets.
- 3. To validate improved feeds and feeding practices on a commercial scale.

Specific strategies for Volume 2: Diet Development:

- 1. To determine digestibility of feed ingredients in order to formulate research and commercial feeds for mulloway
- 2. To identify optimum stocking densities for juvenile mulloway
- 3. To determine optimal protein and energy requirements of rapidly growing mulloway
- 4. To increase understanding of how size and temperature affect the metabolism of mulloway and yellowtail kingfish

The report is presented in two volumes Aquafin CRC – Feed Technology forTemperature Fish Species: Volume 1: Feeding Strategies and Volume 2: Diet Development. The volumes share common background, need, overall objectives, benefits and adoption, further development, planned outcomes, intellectual property and staff. They have individual non-technical summaries, results, discussions and conclusions.

NON TECHNICAL SUMMARY:

World wide, consumption of edible seafood is increasing and by 2025, the world demand for edible seafood is predicted to reach about 140 million tonnes. Capture fisheries are predicted to meet no more than 42% of this demand, with the bulk being met through aquaculture. In fact, world wide aquaculture production has recently achieved parity with wild capture fisheries. The aquaculture of many species, especially the high value carnivorous species most often cultured in the developed world has historically relied on the use of fish meal and fish oil to provide the protein and energy contained in commercial aqua-feeds. These ingredient resources are and will continue to be under considerable pressure and as such they are becoming increasingly expensive. Feed grade ingredients often used in agri-feeds are also being directed into emerging industries such as the bio-fuel sector, further increasing competition and market volatility. Now, more than ever, aquaculture nutrition research is focusing on feed alternatives and ways to improve production efficiencies through a thorough understanding of the nutritional requirements of species. These advances improve the profitability of farms, ensure that consumers have access to high quality, nutritious seafood and that the impacts on the environment are minimised.

The research presented in this report has endeavoured to meet the aforementioned challenges as they pertain to the mulloway and yellowtail kingfish industries in NSW and other parts of Australia. In particular, this volume focuses on requirement studies that have increased our knowledge of the digestible protein and energy needs of mulloway and yellowtail kingfish. This volume also provides valuable information on the digestibility of Australian feed ingredients by each species. Together, this work has resulted in the development of a bio-energetic model for both species which will be of great benefit to farmers and feed manufacturers, allowing construction of feeding tables, greater flexibility in feed formulation and the confidence to utilise alternatives to fishmeal in diets for either species. This report also presents separate studies investigating the utilisation of carbohydrates by mulloway and kingfish and the effects of increasing temperature on fish metabolism. In addition, we present two experiments that elucidate the effects of stocking density on the performance of mulloway during the important juvenile stages of growth.

Evaluation of commercial feeds for mulloway and yellowtail kingfish

Sections 4.1 & 4.11

An 8 week benchmarking study was undertaken at the inception of this project to evaluate the performance of mulloway fed on 6 different commercial feeds. The formulation, ingredient composition and digestibility of the diets was unknown, however, the physical characteristics of the diets and their nutrient compositions provided a range of criteria against which to make preliminary recommendations about their suitability for mulloway grown in cages. Based on the results of this study we do not recommend feeding mulloway on buoyant feeds. In addition, in the absence of other information a sinking feed containing approximately 46% CP, 19.4% lipid, 26% NFE and 8% ash with an overall gross energy density of about 23 MJkg⁻¹ diet appeared to be suitable in terms of optimising weight gain and feed efficiency. Feeding mulloway a diet with elevated crude protein content (51%) and lower lipid (11%) content also promoted satisfactory weight gain and feed efficiency despite recording a slightly reduced feed intake. This may indicate that lipid or carbohydrate have a minimal protein sparing action in diets for this species.

A 42 day study was undertaken to examine the effect of water temperature (16°C and 23°C) on the weight gain and performance of yellowtail kingfish fed six commercially manufactured diets (Winter, GMO-LAP Free, Marine 90238, Marine 90239, Marine 90237, Marine 90235). Diets were similar in crude protein (460 g kg⁻¹), lipid (200g kg⁻¹) and gross energy (22MJ kg⁻¹) content, but the type and ratio of lipids used in each diet was different (i.e. fish oil or poultry oil) and the level of fishmeal in diets varied between 35% and 50% (commercial in confidence). Our results indicated that diet type had no significant effect on weight gain, feed intake, food conversion ratio (FCR) or thermal growth coefficient (TGC). However, elevating the rearing temperature from 16°C to 23°C more than doubled the relative weight gain of yellowtail kingfish (10.6g kgBW d⁻¹ vs 22.5g kgBW d⁻¹), increased relative feed intake by approximately 53% (22.28g kgBW d⁻¹ vs 34.25g kgBW⁻¹ d⁻¹) and improved feed conversion ratio from 2.16 to 1.57. These results demonstrate overwhelmingly that the aquaculture production of yellowtail kingfish will be far more economical at temperatures approaching 23°C. They also demonstrate that substitution of fish oil with different levels of poultry oil or feeding diets that contain different levels of fish meal do not adversely affect the short term performance of yellowtail kingfish reared at either of these temperatures. Assuming a constant water temperature of 16°C and a stocking size of 5g, yellowtail kingfish would take approximately 508 days to reach 2kg. By contrast, if a water temperature of 23°C was maintained they could expect to reach the same body weight in approximately 229 days.

Digestibility of feed ingredients by mulloway and yellowtail kingfish

Sections 4.2 & 4.12

Sections 4.2 and 4.12 describe a series of experiments that determined the apparent digestibility (ADC) of a range of diets and feed ingredients fed to sub-adult mulloway and yellowtail kingfish. Ingredients tested were classified into fish meals, rendered by-product meals, cereal grains, legumes and oilseeds. Apparent digestibility of ingredients was measured by applying an indirect method of determination (marker method; chromic oxide) and collecting faecal material from mulloway by passive settlement. Settlement methods were not appropriate for kingfish and faeces had to be collected by manual stripping. The digestibility of the majority of protein rich ingredients excluding blood meal was determined at a 50% inclusion level. The digestibility of extruded wheat or pre-gelatinised wheat starch was examined at inclusion contents ranging from 10 to 40% of the diet in mulloway, but only the digestibility of extruded wheat was examined in kingfish.

As expected the digestibility of fishmeal by both species was high. As such this ingredient will continue to be a primary protein source in aqua-feeds for these fish and will serve as a benchmark against which other ingredients are measured. Fish oil, poultry oil or canola oil was almost totally digested by kingfish. The protein digestibility of rendered animal meals such as meat meal, poultry meal and blood meal was also high, but digestibility of feather meal was low. The low overall digestibility of feather meal by both species may indicate this batch was subjected to overheating during the rendering process.

Our results demonstrated that protein from extruded wheat was well digested by mulloway and kingfish (>80%) and appears to be independent of inclusion level. However, only about 40-60% of the gross energy in extruded wheat was digested. The digestibility of pre-gelatinised wheat starch by mulloway was significantly better than extruded wheat, but there were stepwise reductions in organic matter and gross energy ADC's of pre-gelatinised starch as inclusion levels were increased from 10 to 30% of the diet. Importantly, our data indicate that ADC's of carbohydrates for mulloway and kingfish are not additive, which has significant implications for feed formulation. The protein in soybean meal, dehulled lupins and whole field peas was also well digested, but the energy digestibility of whole field peas was very poor and reflects the significant amount of carbohydrate (fibre) present in this product. Both mulloway and kingfish were better at digesting dehulled lupin meal than whole field peas, however solvent extracted soybean meal was more fully digested than either of the legumes we tested. Dehulled lupin or solvent extracted soybean meal will serve as useful protein or energy sources in diets for both species. The data we present on the proximate composition of feed ingredients as well as their concomitant digestibility coefficients will serve as a useful starting point for constructing a larger data base of nutritional information required to confidently formulate aqua-feeds for both species. This data base will become more important as the global pressures on fish meal and fish oil resources increase.

Utilisation of carbohydrate by mulloway and yellowtail kingfish

Sections 4.3 & 4.14, 4.15 & 9.6

Utilisation of carbohydrate (CHO) in mulloway and yellowtail kingfish was examined using intraperitoneal or feeding glucose tolerance tests (IP). The injection of 1 g D-glucose kg⁻¹ BW into the peritoneal cavity of mulloway resulted in an immediate and prolonged elevation of plasma glucose concentration. Plasma glucose concentration peaked 6 hours after injection (peak value = 21.9 mM) and remained elevated for up to 48 hours before returning to normal basal levels. This indicated mulloway were intolerant of CHO. In contrast, juvenile yellowtail kingfish injected with the same amount of D-glucose experienced a peak plasma concentration of 12.8mM 1 to 2 hours after injection and remained in a hyperglycaemic state for only 12-18 hours. This indicated that the glycaemic response of yellowtail kingfish was more like that of an omnivorous species such as silver perch than of a strict carnivore such as barramundi. When yellowtail kingfish were fed different doses of Dglucose similar and predictable responses were observed. However, when kingfish were fed diets containing pre-gelatinised starch (PGN) there was no significant increase in plasma glucose concentration. Based on these responses we challenged juvenile kingfish with diets containing 10, 20, 30 or 40% extruded wheat or pre-gelatinised wheat starch. The experiment was run using a summit / diluent approach by which the response of kingfish fed diets containing CHO was compared to fish fed diets containing a similar amount of diatomaceous earth (DE; i.e. inert diluent). Surprisingly, the relative weight gain of kingfish fed CHO diets remained high (17.9-24.3g kgBW^{-0.8}d⁻¹), even at elevated CHO inclusion content. Apart from fish fed diets containing DE and 40% PGN, Relative weight gain of kingfish fed the reference diet and those fed diets containing up to 40% EW or 30% PGN was similar. Feed conversion ratios also remained relatively stable in fish fed diets containing up to 40% EW and 30% PGN (i.e. FCR = 1.3-1.6). Yellowtail kingfish recorded protein efficiency ratios (PER) of approximately 1.6 when fed diets containing EW or 1.7 when fed diets containing PGN, regardless of ingredient inclusion level, indicating that significant protein sparing was occurring. The efficient utilisation of EW and PGN by juvenile yellowtail kingfish reared under the conditions imposed by this experiment indicates that increased levels of dietary CHO and moderate reductions in dietary protein are possible without overly affecting growth performance and protein retention in this species.

Bio-energetic models for mulloway and yellowtail kingfish

Sections 4.6, 4.7, 4.8, 4.9, 4.10 & 4.13

Bio-energetic models were developed for mulloway and yellowtail kingfish. These models describe the growth rate, protein and energy retention, maintenance requirements and changes in the chemical composition of whole carcass for fish weighing up to 2kg. Each model was used to estimate the digestible protein and energy requirements of growing fish and iteratively determine the most appropriate protein and energy content of hypothetical feed formulations. In addition, the models were / can be used to estimate theoretical feed conversion ratios (FCR) and construct feeding tables for either species. These models will be an invaluable tool for feed formulators and will provide farmers with benchmarking data on the potential weight gain, FCR and feed intake they should expect in rapidly growing mulloway and yellowtail kingfish.

In developing these models we also conducted additional experiments on mulloway and kingfish to determine their routine metabolic rates (RMR) at different temperatures. These experiments demonstrated that RMR in both species increased with increasing temperature and that yellowtail kingfish have a RMR which is nearly double the RMR of mulloway. In addition, these experiments were able to establish the temperature at which RMR of either species was least thermally dependant on temperature. These temperatures were found to be 28.5°C and 22.8°C in mulloway and kingfish, respectively and were considered representative of the ideal or optimal temperature for rearing each animal.

Effect of stocking density on performance of juvenile mulloway

Sections 4.4 & 4.5

Two growth studies were undertaken to evaluate the effect of stocking density on the weight gain and performance of juvenile mulloway. These studies showed that weight gain is reduced and feed conversion ratio is increased when the selected stocking density is too low. We concluded that juvenile mulloway weighing approximately 17-60g should be stocked into cages at starting densities exceeding 4 to 6 kg m⁻³ to improve weight gain and FCR. Weight gain and FCR in juvenile mulloway can be optimised at rearing densities approaching 16 kg m⁻³. Higher stocking densities may be plausible but these may result in poorer FCR. The worsening in FCR may be due to the inherent difficulty in hand feeding a large number of fish to apparent satiation. This problem would tend to be exacerbated 'onfarm' thus some form of automated feeding system may be required. Small mulloway show no preference for feeding in the morning or the evening, at least under the conditions we tested giving farmers of juvenile mulloway the confidence to feed their fish at either time of the day.

KEYWORDS:

Mulloway; yellowtail kingfish, digestibility, ingredients, protein requirements, bioenergetics, metabolism, stocking density

Farming of marine fish in Australia is continuing to develop and is principally based on seacage growout of tuna (*Thunnus maccoyii*) and yellowtail (*Seriola lalandi*) in South Australia (SA) and Atlantic salmon (*Salmo salar*) in Tasmania. Production of yellowtail in SA was reported as 50 and 700 t in 2000/01 and 2001/02 respectively, and predicted to reach 1500 t in 2002/03 (Hutchinson, 2003). Production of mulloway (*Argyrosomus japonicus*) in South Australia has recently increased as farmers have sought an alternative species to culture following problems with yellowtail culture (the major problems include skin and gill flukes). In NSW, snapper (*Pagrus auratus*) mulloway, and silver bream (*Acanthopagrus australis*) are also being farmed in small quantities for domestic consumption. Small numbers of other estuarine species, including black bream (*Acanthopagrus butcheri*) sand whiting (*Sillago ciliata*) Mahi mahi (*Coryphaena hippurus*) and eels (*Anguilla australis* and *A. reinhardtii*), have been produced intermittently for research and or commercial evaluation in various states. Estimated combined production for these species in Australia for 2002/03 is close to 140t. A large marine fish farming venture in Morton Bay, Qld is seeking approval but facing considerable local opposition.

Industry interest in temperate marine species is obviously driven primarily by economics but also by available technology, particularly for fingerling production and diets. Farmers in South Australia and NSW focused initially on snapper because hatchery technology was proven (although costs of fingerlings were very high) and attractive initial market prices. In SA, farmers turned from snapper to mulloway and then yellowtail following poor market prices for snapper (exacerbated by unattractive, dark skin colour) and relatively slow growth rates. Yellowtail was the species of choice in 2001 and 2002 because of rapid growth rates for this species and initial high market price. However, increasing problems with parasitic flukes, reducing market price and localised opposition to yellowtail farming encouraged the farmers to either shift back to mulloway or at least consider other species. The reduced market price for yellowtail was an inevitable consequence of increasing production but was also fuelled by the increasing value of the Australian dollar and the consequent reduction in export value and increasing competition from imports on the domestic market.

In NSW, farmers also started with snapper and although they also struggled with market acceptability due to skin colour, their proximity to the market made this easier to overcome. Availability of sufficient numbers of fingerlings restrained production and, at least for one farm, parasite problems were experienced. Mulloway and silver bream have been trailed as alternative species.

This shift between species is likely to remain a feature of Australian temperate marine fish culture for several reasons. Firstly, the current small demand has meant hatchery development has been slow and inherently risky. There are only a small number of hatcheries and this increases the risk that production of any particular species will fail. Having more than one species to breed, especially if natural breeding seasons are offset in time, reduces the risk of failure. Secondly, the small domestic market in Australia is particularly prone to supply driven price decreases. Having several species available for sale mitigates this problem to some extent. Thirdly, at this stage in the industry, there is simply not enough industry experience to determine if one particular species is most suited to large-scale culture. Farmers still need the flexibility to experiment with a number of species to determine which ones suit their particular operation.

Technology for snapper has progressed rapidly following the Aquafin CRC project 1B.3-2001/208 (Increasing the profitability of snapper farming by improving hatchery practices and diets). This project has significantly reduced both hatchery and diet costs. Two species currently have the most potential (and

industry interest) as alternatives: yellowtail and mulloway. Yellowtail grows rapidly and is well suited to culture in sea cages. However, in SA, high mortality rates have been experienced following infestations of the skin fluke (*Benedenia seriolae*) and the gill fluke (*Zeuxapata seriolae*). These organisms have also caused serious mortalities in Japan where approximately 100,000 t/yr of yellowtail are grown.

Although available "marine fish" diets have produced rapid growth rates, some farmers and feed manufacturers have questioned the nutritional adequacy of available diets, especially for rapidly growing fish. Slow growth, especially over winter, together with mortality unrelated to parasites, has also been attributed by some farmers to inadequate nutrition.

Mulloway were first bred in Australia by the team at PSFI in NSW in 1992. This species has considerable aquaculture potential due to its almost Australian wide distribution and fast growth rates. Commercial grow-out of this species is occurring in NSW and SA but the combined production in 2002/03 was only several hundred tonnes. Interest in mulloway in southern states initially declined in favour of yellowtail but as mentioned above, has made a resurgence over the last year. Mulloway appears to be extremely hardy and very few problems with parasites or diseases have been recorded, despite quite extensive farming trials in NSW and SA. The potential for grow-out of this species is not limited to seacage culture. Due to its extremely euryhaline nature (5-35ppt) the species has potential for estuarine pond culture and recent grow-out trials in both NSW and SA inland saline water bodies have been promising. The gregarious nature of this animal may allow culture at quite high densities and use of recirculating aquaculture systems. One notable difference between mulloway and kingfish is their feeding behaviour. Kingfish feed actively on the surface while mulloway are sub-surface feeders. Changing buoyancy of feeds is possible using extrusion technology but can restrict ingredient choice.

Yellowfin bream (*Acanthopagrus australis*) are currently being grown by one farmer (Silver Beach Aquaculture) in Botany Bay who has indicated the fish are performing quite well and are exhibiting similar growth rates to the closely related snapper. Prices paid for bream are similar and can be better than that paid for snapper and fingerlings are available from northern NSW hatcheries.

INDUSTRY CONSULTATION

The research proposed in this project was listed as a priority area for research by the Aquafin CRC Joint Management Advisory Committee, the Yellowtail Kingfish Aquaculture Strategic R & D Plan 2003-2008 and at the Aquafin CRC Snapper Workshop (Allan 2003). It has been requested by marine fish farmers in NSW and SA and by the largest feed manufacturer in Australia (Ridley Pty Ltd). The priority placed on the research by industry is clearly demonstrated by an industry commitment of over \$235,610 in cash to the research.

AQUAFIN CRC – PROJECT DEVELOPMENT AND PRIORITY

This project is the second of two projects I & I NSW Fisheries is leading under the Aquafin CRC. I & I NSW signed up as a participant in the Aquafin CRC (2001-2008) to develop technology for temperate marine finfish farming. The first Aquafin CRC project 'Increasing the profitability of snapper farming by improving hatchery practices and diets' (R-Aqua2001/029) was agreed to after extensive consultation with marine finfish farmers in NSW and SA. At the time the project was initiated, both major commercial farmers in NSW were collaborators and both are still participants of the Aquafin CRC. This second project has also been developed following extensive consultation with marine finfish farmers in NSW, Qld, WA and SA. A workshop was convened in Melbourne in September 2002 (Allan, 2003) where priorities for future research on temperate marine finfish were discussed. This project has arisen following these consultations. During the development phase for this project, individual marine fish farmers in SA

and NSW, the South Australian Marine Finfish Farmers Association (SAMFFA), Ridley Aquafeeds Pty Ltd, the FRDC Aquaculture Nutrition Subprogram and, of course, all collaborating research providers were consulted. The Yellowtail Kingfish Aquaculture R&D Plan (2003-2008), lists as highest priority fish health issues, and "next generation technologies" (objective 2 of this application addresses these issues), as high priority stress management (addressed by objective 2) and as medium priority nutrient impacts and carrying capacity (addressed by objective 2, particularly bioenergetic models). Strategic research issues to improve production efficiency and reduce costs were listed as "becoming increasingly important over time". The research in this application will build the foundation for these efficiencies and cost savings through improvement in grow-out diets and feeding technologies. Although initially very supportive, the SAMFA decided they were unable to commit cash to this project, partly because of the long-term nature of the project. Even so, outcomes from the project will be of significant benefit to SA marine fish farmers. Specifically, diets developed and adopted by the industry partner, Ridley Aquafeed Pty Ltd, will be available to SA farmers and SA farmers will be consulted about evaluation of project diets. Every effort will be made to involve them in feeding trials. Secondly, costs of fingerlings are very high in SA. Even though commercial farmers in SA do not list R&D to reduce fingerling production costs as a high priority, commercial fingerling costs are excessive (e.g. >\$2.00/fingerling for mulloway). This project will reduce costs of fingerling production. (As an example of previous success, costs for snapper fingerlings were reduced by 30% as a result of R&D on a previous Aquafin CRC project carried out by the current project team). The methodology described in this application is consistent with recommendations that have arisen from the Aquaculture Nutrition Subprogram workshops. This project was accorded priority from both CRC and FRDC boards (with suggested revisions in light of the SAMFA decision to reverse their support). The suggested revisions have all been made although budget is slightly larger (13.7%) than recommended by the FRDC Board.

2. NEED

Temperate marine fish farmers in Australia seek to have the option to choose between several species of fish based on market price, availability and cost of fingerlings and health and feeding costs. This need was identified at the Aquafin CRC workshop held in 2002 (Allan, 2003). Most research on temperate marine finfish has been conducted on snapper (*Pagrus auratus*) but farmers are now concentrating more on yellowtail (*Seriola lalandi*) and mulloway (*Argyrosomus japonicus*) with significant interest in yellowfin bream (*Acanthopagrus australis*). Applied research is needed, particularly on the major cost areas of diets and feeding (for both fingerlings and grow-out fish) to help ensure profitability and to give farmers and feed manufacturers information so they can make informed business decisions.

Costs of feeds and feeding are usually the largest budget expense for marine fish farms and also significantly affect costs of producing fingerlings in hatcheries. In hatcheries, the global shortage of Artemia and the huge cost of weaning diets have led to a increased priority for better and cheaper live feeds, formulated weaning diets and feeding strategies. For grow-out, most farmers want highperformance, low-cost feeds. Given a choice, most farmers will pay more for diets to achieve better performance but have no real way to make decisions to achieve the most cost effective feeding strategy. There is a clear lack of information for most temperate marine species about the nutritional specifications needed for high performance diets and what physical characteristics are most desirable in the pellets (e.g. should pellets be floating or sinking, how important is pellet hardness, etc). This prevents feed manufacturers providing data-based recommendations about the best diets for farmers and prevents them from formulating and manufacturing specific diets for temperate marine finfish farmed in Australia. Unfortunately, the same lack of information is restricting choices about the ingredients being used in diets. Almost no information exists about digestibility or utilization of most of the ingredients available for use in Australia aquafeeds. Most farmers are aware of "problems" with the use of terrestrial animal protein meals in animal feeds and that use of such ingredients might negatively affect the export market for their fish. However, apart from research with snapper (Aquafin CRC; WA Fisheries) and barramundi (FRDC ADD Subprogram; WA Fisheries) there is no information about digestibility or utilization of Australian ingredients for temperate marine finfish being farmed in Australia. The immediate result of this lack of information is an increase in the proportion of expensive, imported fishmeal being used in diets.

This project will extend the successful research approach adopted for snapper in Aquafin CRC Project 1B.3-2001/208 (Increasing the profitability of snapper farming by improving hatchery practices and diets). In that project, fingerling costs were reduced by approximately 30% through systematic research to develop more cost-effective hatchery procedures including the demonstration of the feasibility of replacing live feeds including artemia with alternative live feeds (copepods) and/or commercially available, inert pellet diets for advanced snapper larvae. Previous work with snapper also demonstrated a major improvement in growth of juvenile snapper when the optimal feeding frequency and day-length were identified. This project seeks to reduce feed costs, to optimise feeding efficiency and to improve fingerling survival and growth of mulloway and vellowtail. Sub-optimal performance of marine fish larvae is often a result of inadequate nutrition or sub-optimal physico-chemical variables during larval rearing. A high percentage of slow-growing or stunted fish in larval rearing runs can seriously reduce economic viability of hatcheries and increase farming costs. The performance of larvae has not been addressed in a systematic manner and although the commercial hatcheries in SA report that fingerling production is not a barrier, there are no published methods of how to optimize production of fingerlings (i.e. to increase cost-effectiveness of fingerling production). This lack of information will reduce the chance of expanding marine fish farming in NSW and other states in Australia.

Existing grow-out diets used for marine fish such as yellowtail, mulloway and bream are based on generic formulations for "marine fish" (including salmon and barramundi). These diets produce results but it is unknown if current diets are nutritionally adequate, especially for rapidly growing fish. Even basic requirements, like the best protein to energy ratio, are unknown for yellowtail and mulloway. Both low and high energy diets are available for salmon and barramundi but even simple comparisons to find the best of these two "options" have not yet been carried out. There is no reliable information on ingredient digestibility making it impossible for feed manufacturers to confidently formulate diets with alternative protein sources to fishmeal when fishmeal is hard to obtain and when prices are high (and, of course, fish meal prices continue to rise). Research to provide this information is urgently needed.

There are obvious problems with a "one-species at a time" approach to diet development research. This is expensive and takes a long time. This application seeks to conduct specific research with mulloway and kingfish and to build comprehensive models of nutritional requirements for these two species that can be directly compared with other similar models now available for other marine and freshwater aquaculture species (e.g. snapper, sea bream and barramundi).

Fingerling costs for mulloway and kingfish are currently estimated at \$0.60->\$2.00/fingerling. These represent well in excess of 10% of operating costs. We aim to reduce these costs by as much as 50%. Growout feeds can cost in excess of \$2,000/t and costs of feeding are usually in excess of 30% of total operating costs (>50% for some operations). Food conversion ratios of in excess of 1.5:1 are regularly reported. We aim to produce diets with FCRs of 1.2:1 with approximately 25% lower ingredient costs. Together these represent the major areas where improvements in production technology can improve the profitability of marine fish farming.

3. OBJECTIVES

OVERALL OBJECTIVES:

- 1. To reduce costs of fingerling production.
- 2. To improve the cost-effectiveness of grow-out diets.
- 3. To validate improved feeds and feeding practices on a commercial scale.

Specific objectives for this Volume 2: Diet Development:

- 1. To determine digestibility of feed ingredients in order to formulate research and commercial feeds for mulloway
- 2. To identify optimum stocking densities for juvenile mulloway
- 3. To construct and test factorial models based on the protein and energy requirements of rapidly growing mulloway
- 4. To increase understanding of how physico-chemical parameters such as temperature affect the metabolism of mulloway and yellowtail kingfish

4. **RESULTS AND DISCUSSION**

4.1 Weight gain and performance of juvenile mulloway *Argyrosomus japonicus* reared in floating cages and fed commercially manufactured feeds.

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1. INTRODUCTION

Several commercial diets are available for farmers of temperate marine finfish in Australia. These diets are commonly fed to barramundi, Atlantic salmon, snapper, mulloway and more recently yellowtail kingfish. Some of the available diets are specifically formulated for one species (e.g. A. salmon or barramundi), and are therefore probably not ideal for other temperate fish in terms of nutrient content, nutrient balance or ingredient composition. This dilemma is common in aquaculture, where many new fish are being evaluated and dietary requirements are poorly understood. Therefore, farmers which diversify into new species are basically constrained to feeding these animals diets that are commercially available and affordable. The goal then becomes to feed the diet that as far as possible matches the perceived or best guess nutritional requirements of the species until more information is available. The first step in such an investigation is to evaluate or benchmark the weight gain and performance of the new species on a range of readily available commercial diets. This research gives invaluable insights into the gross nutritional requirements of a species by indicating which of the available diets promote better growth and feed conversion. Different diets may also differ in pellet characteristics such as bulk density which affects buoyancy and is known to affect voluntary feed intake (Booth, Allan & Warner-Smith, 2000; Booth, Allan, Evans & Gleeson, 2002). Thus matching the physical quality of the pellet to the feeding behaviour of a new aquaculture species can be as important as the nutrient profile of the diet.

The aim of this experiment was to compare the growth and feed conversion of juvenile mulloway fed six commercially manufactured feeds. All feeds were produced using extrusion technology and varied in crude protein, lipid, carbohydrate and gross energy content. In addition, each of the feeds varied in terms of bulk density, ranging from floating to rapidly sinking pellets.

2. MATERIALS AND METHODS

2.1 Diets and pellet manufacture

Four of the tested feeds were made at the Australasian Experimental Stock-feed Extrusion Centre (AESEC; University of Adelaide, Roseworthy Campus, South Australia). For these feeds, approximately 800 kg of mash was dry mixed and finely ground (200 um) by staff at Ridley Aquafeed Pty. Ltd. (Narangba, Qld, Australia). Afterwards the dry mash was shipped to the AESEC for pellet manufacture. Extruded pellets (6 mm diameter – short cut) were manufactured in a Wenger X-85 mini mill and dried in associated Wenger dryers and coolers. Fish oil was added in the pre-conditioner or by post-pellet vacuum coating in a UAS vacuum infusion system. Dried pellets were stored in hermetically sealed bags (\approx 15 kg) after cooling and then freighted to PSFI where they were stored at < -15°C. These feeds were labelled D2,

D3, D4 or D5, respectively (Table 1). The other two commercial feeds were obtained directly from Ridley Aquafeed and were considered representative of aquafeeds currently being used by industry. These feeds were labelled COM A and COM B, respectively. The formulations of all feeds were commercial inconfidence.

2.2 Fish and stocking procedures

Mulloway used in this experiment were progeny of brood-stock held at the NSW DPI Port Stephens Fisheries Institute (PSFI). Prior to the experiment they were held in large 10 kL tanks and fed a sinking barramundi feed (typical analysis 43% crude protein; 20% crude fat; 2.5% crude fibre; Ridley Aquafeed Pty Ltd, Narangba, Qld, Australia). Fish were always anaesthetised (15-30 mg ethyl-p-amino benzoate L⁻¹) prior to weight check or handling procedures.

Twenty-five juvenile mulloway (mean individual weight \pm stdev; 128.5 \pm 7.4 g) were stocked into cylindrical experiment cages made from 10 mm perforated plastic mesh (200 L). Each cage was lined with a black vinyl insert that prevented the loss of floating pellets from the surface while the base of each cage was fitted with a feeding mat (3 mm perforated plastic mesh) that prevented the loss of sinking pellets. Each cage was also fitted with floatation aids and secured to the perimeter of a circular 10 kL fibreglass tank (3.4 m diameter; 1.1 m deep). These 10 kL tanks were located inside a shade-house at PSFI and formed part of a larger saltwater recirculation system. Six cages were secured in each of the 10 kL tanks (total of 36 cages).

Fish were fed to apparent satiation at approximately 0900 h and 1500 h from Monday to Saturday. Fish were fed only once on Sunday (0900 h). Initially, all mulloway were fed a cocktail mix of the six commercial feeds for the first 4 days of the experiment. After this period each of the commercial diets was randomised to one of six experiment cages and fish were fed their respective diets for the remainder of the trial. Any uneaten feed was collected, dried to a constant weight and subtracted from the total feed input of each tank in order to accurately determine feed intake. Fish were not fed the day before weight check procedures and fish that died were not replaced. The trial was run for 72 days.

Water quality was maintained at acceptable levels by vacuuming each of the 10 kL tanks at least twice weekly and exchanging small but regular volumes of water. Each tank was heated using a 2.4 kw immersion heater in order to maintain water temperatures $> 20^{\circ}$ C and aerated using compressed air forced through a combination of small and large submerged air stone diffusers. The flow rate to each tank was set at approximately 30 L min⁻¹.

Water quality parameters were recorded daily using a hand held water quality instrument (Model 611; Yeo-Kal Electronics, Brookvale, NSW, Australia). During the experiment the mean \pm sd of temperature, dissolved oxygen (DO₂), salinity and pH were 22.7 \pm 1.4°C, 6.0 \pm 0.8 mg L-1, 33.4 \pm 3.8 and 8.0 \pm 0.3 units, respectively. Total ammonia [NH₃ + NH₄⁺] was monitored using a rapid test kit procedure (Model 1.08024.0001, E. Merck, Darmstadt, Germany) and was always \leq 0.6 mg L⁻¹.

2.3 Chemical analyses

The chemical composition of feed samples was determined by two providers: Industry and Investment NSW Diagnostic Services Unit (Wagga Wagga Agricultural Institute, Wagga Wagga, NSW, Australia; NATA accredited) and Queensland DPI&F (Health & Nutritional Biochemistry Unit, Moorooka, Qld, Australia). All analyses were conducted according to standard (AOAC, 1990) or in-house analytical techniques.

2.4 Statistical analyses

One experiment cage randomised to diet D2 and one cage randomised to diet D5 was lost during the experiment. Minor mortality was recorded in 9 other cages assigned to diets COM A, COM B, D2 or D4 (i.e. 1 to 5 fish per cage; 16 mortalities in total). For these cages, average response variables were calculated from the number of fish remaining at harvest.

The effect of different commercial diets on specific harvest criterion of juvenile mulloway was analysed using one way analysis of variance (ANOVA). Data were statistically analysed using StatGraphics Plus (StatGraphics Plus for Windows, Version 4.1, Statistical Graphics Corporation). Prior to ANOVA, raw data were checked to ensure homogeneity of variances (Cochran's test) and normality. Outliers more than 1.5 times the inter-quartile range were identified in exploratory data analysis. One replicate data point was identified in treatment D5 affecting harvest weight and SGR. Mulloway from this replicate performed more poorly than others in this group and it was removed from all statistical analyses (n=4). Four outliers were identified in plots of feed efficiency, two each in treatments D2 and D4, respectively. In each case there was one outlier located above and below the box. Removal of the lower outlier was sufficient to normalise the spread of data with respect to diet D2, while both outliers were removed to normalise the spread of data about the median in diet D4. In addition, raw data for per cent survival was arcsin-square root transformed in order to satisfy the assumptions of ANOVA. The significance level for ANOVA and multiple comparisons tests (*Tukey HSD* test) was set at 0.05. All figures and iterative models were produced using GraphPad Prism 4 for Windows (GraphPad Prism Software, Inc) or NCSS (Hintze, 2006).

3. **RESULTS**

There was no significant difference in the survival of mulloway after 72 days ($F_{5,28} = 1.30$; P=0.2928). However, there were highly significant differences detected between diets for the response criteria of harvest weight ($F_{5,27} = 8.37$; P=0.0001), individual weight gain ($F_{5,27} = 8.73$; P=0.0001), specific growth rate (SGR) ($F_{5,27} = 9.36$; P=0.0001), relative feed intake ($F_{5,27} = 9.33$; P<0.0001) and feed efficiency (FE) ($F_{5,24} = 6.91$; P=0.0004). Mean performance criterion of mulloway are presented in Table 2. The pattern of response was identical for harvest weight, individual weight gain and SGR; in each case mulloway fed the COM A diet were significantly smaller at harvest and grew more slowly than fish fed all other treatments (Table 2). Despite the sub-opitmal growth of fish fed COM A, the growth rates of mulloway fed on the other diets was consistent with growth rates determined for juvenile mulloway in other studies at PSFI (Figure 1). Data recorded for FE was more variable and was significantly lower in diets COM A and D3 than in diets COM B and D2 (Table 2). There were significant differences between the relative feed intake of fish fed diet COM A and D3, however discrete groups were not clearly identified. During the trial we observed that mulloway were somewhat reluctant to feed on the more buoyant feeds (i.e. COM A and D2). This behaviour has clearly impacted on the performance of these fish, particularly those fed on COM A (100% floating pellets) and is reflected in the reduction in SGR, feed intake and FE of fish fed this diet (Table 2). Further examination of the data excluded this treatment.

There were marked differences in the gross nutrient composition of the remaining 5 diets. Of the macro nutrient classes the most dramatic differences were among crude protein (CP), lipid (CL) and NFE contents. Only minor differences were measured in gross energy (GE) and ash content (Table 1). Numerically, the highest SGR and best FE was recorded in mulloway fed sinking diet COM B, which had a CP, CL and NFE content of 46.3, 19.4 and 26.3%, respectively.

It was apparent from the data that mulloway growth and FE was best on diet COM B. However, there were minor numerical differences in feed intake among groups that combined with dietary composition may also have impacted on SGR. As such, response surface diagrams were used to explore the effects of

relative nutrient intake (i.e. g nutrient kg BW^{-0.8} d⁻¹ or kJ kgBW^{-0.8}d⁻¹) on SGR of mulloway. Each of the contour graphs clearly identified peaks or "optima" in SGR that occurred when the relative intake of CP and GE were approximately 1.90 g kgBW^{-0.8}d⁻¹ and 95 kJ kgBW^{-0.8}d⁻¹, respectively (Figure 2a). Specific growth rate was also highest when a similar intake of CP was matched with a lipid intake of 0.78 g kgBW^{-0.8}d⁻¹ (Figure 2b) or a NFE intake of 1.10 g kgBW^{-0.8}d⁻¹ (Figure 2c).

DISCUSSION

The aim of this study was to compare the growth, feed intake and FCR of juvenile mulloway fed a range of commercially manufactured feeds in order to make preliminary recommendations to industry. Each of the six feeds was manufactured using extrusion technology which resulted in feeds having different pellet buoyancies. Each of the six diets also differed to a greater or lesser extent in gross nutrient or energy composition with major differences in dietary crude protein, lipid and NFE content (Table 1).

The results have indicated that the growth rates and feed intake of juvenile mulloway can be affected by the physical quality of the feed (e.g. buoyancy) or its nutritional characteristics. For example, diet COM A was almost 100% buoyant and was poorly accepted by mulloway stocked at the densities used in this study and when housed in 200 L floating cages. As a consequence, these fish were difficult to feed to apparent satiation and failed to reach the feed intakes and growth rates of fish fed the other dietary treatments. Diet D2 was semi buoyant and although the feed intake of fish fed this diet was somewhat reduced, their growth rate and FE compared favourably with D3, D4, D5 and COM B. Based on these results we recommend that juvenile mulloway weighing between 100 to 200 g should be fed a sinking diet with a gross nutrient composition that closely matches that of diet COM B. This should result in satisfactory growth rates and efficient conversion of feed. A diet such as D2, with a higher crude protein and lower lipid content respectively than that of diet COM B may be an option if it can be manufactured to sink. However, these recommendations are preliminary and take no account of the digestibility of nutrients or energy in each of the diets or of their ingredient composition.

Dietary protein and energy recommendations for sciaenid's have been made by others. Daniels & Robinson (1986) investigated the effect of dietary protein and energy levels on juvenile red drum, a sciaenid species closely related to mulloway. They indicated that diets containing 35% protein and 17 MJ kg⁻¹ were adequate for good growth and body composition (i.e., low fat and high protein) in red drum reared at 22-26°C. Red drum reared at 26-33°C grew best when fed to satiation on a 44% protein diet at dietary energy levels of 15.4 to 17.2 MJ kg⁻¹ diet. These requirements were reported for fish grown in low salinity water (<7‰) and are lower than the 50% CP recommendations made by an earlier study rearing red drum in seawater (Lin & Arnold, 1983). Optimal weight gain, feed efficiency and protein efficiency ratio was achieved in juvenile giant croaker reared in seawater when they were fed semi-purified diets containing 45% protein (Lee, Cho, Lee & Yang, 2001).

Interest has also focused on the ability of red drum to preferentially utilise lipid or carbohydrate (dextrin) in aquafeeds Highest growth and feed efficiency were observed in red drum fed diets containing 40 to 45% protein and 10% lipid and they were reported to utilize dietary lipid more efficiently than carbohydrate (Serrano, Nematipour & Gatlin, 1992). McGoogan & Gatlin (1999) offered red drum diets containing 35, 40 or 45% crude protein with two different CP: digestible energy ratios (28 vs 22 g CP MJ DE⁻¹). Diets were fed to fish at a rate approaching apparent satiation. They found weight gain of fish significantly increased in response to increases in dietary protein, but within protein level, weight gain was reduced by higher dietary energy content. Increased dietary energy appeared to have beneficial effects at reduced levels of feed intake as reflected by improved weight gain and FE. In a later study these authors fed groups of small red drum a fixed dietary amount of CP (45%) while increasing dietary energy density from about 15 to 18 MJ kg⁻¹ using fish oil, but found no beneficial effect on weight gain or FE

(McGoogan & Gatlin, 2000). Turano, Davis & Arnold, 2002 evaluated the response of sub-adult red drum (186g) to shifting CP:DE ratios by feeding diets containing different levels of CP (360 or 440 g CP kg⁻¹ diet), dietary lipid (83, 103, 123, 143, or 163 g lipid kg⁻¹ diet) and carbohydrate (wheat starch 100-300 g kg-1 diet). They found no significant differences in final weight, weight gain, feed efficiency, protein conversion efficiency or hepatosomatic index after 12 weeks, however intra-peritoneal fat tended to increase with increasing lipid content of diets. They also found that fish offered diets with 440 g CP kg⁻¹ diet produced significantly higher growth and FE values compared to fish receiving diets containing 360 g CP kg⁻¹ diet and concluded that sub-adult red drum were highly tolerant of shifts in CP:GE ratios and were capable of utilising a wide range of dietary lipid and carbohydrate without compromising growth. This conclusion is different to that made by Ellis & Reigh (1991) who found that weight gain, FE, protein retention and energy retention of red drum was inversely related to dietary energy level and dietary carbohydrate, at all energy levels they tested.

The values cited by the aforementioned authors reflect the range of dietary nutrient and energy values measured in the six diets tested in the present study. However, unlike many of those studies we identified no clear relationships between the gross nutrient or energy content of our test diets and performance criteria. This is not unexpected because in most of the previous studies the ingredient composition was tightly controlled or limited and often only a single source of protein or energy was used to manipulate nutrient or energy composition. In some cases the estimation of digestible nutrients or energy was also predicted from physiological fuel equivalents rather than measured directly.

In order to gain a greater understanding of the combined effects of feed intake and gross nutrient or energy content of the commercial feeds, we modelled nutrient intake using response surface diagrams. According to these diagrams SGR was maximised when crude protein and energy intakes ranged between 1.8-1.95g $CP kgBW^{-0.8}d^{-1}$ and 87.5-97 kJ kgBW^{-0.8}d⁻¹, respectively. The ratio of protein to energy intake represents the dietary balance of CP:GE required to maximise SGR (i.e. 20 g CP MJ GE⁻¹). These figures also represent the level of intake required for maximum growth at our experimental temperatures and are similar to DP requirements for maximum protein gain given for gilthead seabream (1.25 g DP kgBW^{-0.7}d⁻¹; Lupatsch, Kissil, Sklan & Pfeffer, 2001) and European sea bass reared at 23°C (2.5g DP kgBW^{-0.69}d⁻¹; Lupatsch, Kissil & Sklan, 2001). Dietary recommendations based on nutrient and energy intake per unit of body weight have also been made for small red drum (50g body weight) grown at 26°C (McGoogan & Gatlin, 1998; Gatlin III, 2002). When converted to the same units as used in our study, their data predict DP requirements for maximum gain as 3.4 g DP kgBW^{-0.8-1}d⁻¹ and 250 kJ kgBW^{-0.8}d⁻¹. These values are slightly higher than those derived from our response surface graphs and probably reflect differences in diet composition, feeding strategy and temperature of their study compared to ours. There is limited information available on the metabolic requirements of mulloway for lipid and NFE, however our interpretation of the data from this study indicates that SGR is maximised when the aforementioned protein intake is paired with a lipid or NFE intake approaching 0.8 or 1.1 kg BW^{-0.8}d⁻¹, respectively.

This study represents the first evaluation of mulloway fed a limited range of commercially manufactured feeds available to marine fish species in Australia. Ingredient composition and digestibility of the diets was unknown. However, the physical characteristics of the diets and their nutrient compositions provided a range of criteria against which to make preliminary recommendations about their suitability for mulloway. Accordingly, we do not recommend feeding mulloway on buoyant feeds. In addition, in the absence of other information a sinking feed containing approximately 46% CP, 19.4% lipid, 26% NFE and 8% ash with an overall gross energy density of about 23 MJkg⁻¹ diet appears to be suitable in terms of optimising weight gain and FE. These recommendations will be superseded once data on the digestibility of a range of ingredients is determined specifically for mulloway and combined with bio-energetic models

that accurately predict the digestible protein and energy requirements for this species over the whole growth cycle.

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TABLE 1

Measured composition of commercially manufactured feeds (dry matter basis)

			Dry	Ash	Nitrogen	Crude	Gross	Fat	NFE
Diet type		Bouyancy	Matter (%)	(%)	(%)	(%)	energy MJ kg ⁻¹	(%)	(%)
D2	n=4 sem	50% sink	95.10 0.45	11.4	8.14	50.89 0.16	21.03	10.73	27.03
D3	n=4 sem	sinking	94.80 0.34	8.7 0.1	7.25 0.14	45.31 0.87	22.01 0.20	13.78	32.19 0.73
D4	n=4 sem	sinking	94.38 0.76	8.7 0.1	6.41 0.01	40.09	22.98 0.21	19.03 0.33	32.21 0.36
D5	n=4 sem	sinking	95.75 0.53	10.1 0.7	7.86 0.07	49.14 0.45	23.46	22.83 0.29	17.94 0.80
COM A	n=2 sem	floating	92.75 0.35	11.1	8.54 0.34	53.34	21.32	11.25	24.31
COM B	n=2 sem	sinking	92.75 0.05	8.0 0.0	7.42 0.12	46.34 0.72	23.0 0.04	19.4 0.8	26.26 0.08

TABLE 2

Mean performance criterion of juvenile mulloway measured at harvest.

Diet type		Survival weight (%)	Harvest gain (%)	Weight (g/fish)	SGR intake (%)	Feed efficiency % BW d ⁻¹	Feed
D2		98.4	176.5 ^b	48.2 ^b	0.44 ^b	0.50 ^{ab}	0.97 ^b
D3		100.0	170.3 ^b	41.4 ^b	0.39 ^b	0.62°	0.63 ^a
D4		98.7	175.8 ^b	47.2 ^b	0.43 ^b	0.58^{bc}	0.76^{ab}
D5		100.0	177.4 ^b	48.6 ^b	0.44^{b}	0.59^{bc}	0.75^{ab}
COM A		96.7	153.3 ^a	24.8^{a}	0.24^{a}	0.42^{a}	0.57^{a}
COM B		95.3	184.8 ^b	57.0 ^b	0.51 ^b	0.59 ^{bc}	0.86 ^b
sem	n=4	2.1	4.5	4.5	0.035	0.029	0.06
sem	n=5	1.9	4.0	4.0	0.032	0.027	-
sem	n=6	1.7	3.7	3.7	0.029	0.024	0.05

N.B. Survival data was transformed prior to ANOVA. All values in the table are untransformed data.



FIGURE 1

Change in specific growth rate of juvenile mulloway with respect to changes in average body weight (data collated from different experiments conducted at PSFI). Relationship described by a power function $Y=A \cdot BW^{b}$; SGR=6.578 (±2.382) x BW^{-0.462(±0.088)}, R²=0.79.



FIGURE 2a

The effect of relative crude protein and gross energy intake (g kgBW^{-0.8}d⁻¹ and kJ kgBW^{-0.8}d⁻¹) on specific growth rate of juvenile mulloway.



FIGURE 2b

The effect of relative crude protein and lipid intake (g $kgBW^{-0.8}d^{-1}$) on specific growth rate of juvenile mulloway.



FIGURE 2c

The effect of relative crude protein and NFE intake (g $kgBW^{-0.8}d^{-1}$) on specific growth rate of juvenile mulloway.

4.2 Digestibility of selected feed ingredients for mulloway Argyrosomus japonicus

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1. INTRODUCTION

The sea cage aquaculture of mulloway *Argyrosomus japonicus* in Australia is conducted at protected sites predominantly in NSW and South Australia (SA). Aquaculture production volumes remain fairly low (several 100 tonnes combined; ABARE 2007), however there is a great expectation that both state industries will continue to expand to meet growing local and export demand for this species. Sea cage industries for this species may be complemented by aquaculture ventures located at inland saline groundwater interception schemes (Fielder, Bardsley & Allan, 2001).

A temperate marine carnivore, mulloway possess many of the attributes thought to be important for a successful aquaculture species such as a wide tolerance of temperature and salinity (5-35°C & 5-40ppt), reasonable growth rates, resilience to disease and tolerance of high stocking density (Silberschneider & Gray, 2008). They are targeted by sport fishers and are well accepted by consumers. Mulloway are thought to have as much aquaculture potential as others in the same genus (i.e. red drum *Sciaenops ocellatus*). At present, farmed mulloway are grown on commercial aqua-feeds formulated for Atlantic salmon *Salmo salar* and barramundi *Lates calcarifer*.

Industry and researchers have identified several factors that will limit the expansion and profitability of the mulloway farming (i.e. Aquafin CRC). Key constraints include a lack of knowledge about basic nutritional requirements, optimal diet specifications, digestibility of ingredients, feeding protocols and the effects of environmental parameters such as temperature and salinity on feed intake and nutritional physiology. Collectively, the aforementioned factors represent different but important components of diet development research.

As for most new species, one of the first critical steps in diet development research is the determination of apparent digestibility coefficients (ADC) for a range of potential feed ingredients. Determination of ADCs is important for several reasons. Firstly, it is extremely useful for indicating the nutritional potential of a feed ingredient. Secondly, it permits formulation of research diets on a digestible nutrient basis because undigested nutrients and energy are accounted for. This allows more rigorous evaluation of feed ingredients and of their prospective inclusion levels, because diets can be compared on a similar digestible protein and energy basis. Thirdly, the uptake of this information by commercial feed companies ensures that the dietary specifications of their proprietary feeds are as consistent as possible. This is particular important where diets are formulated on a least cost basis and component feed ingredients are constantly varied depending on price or availability. Formulating more highly digested feeds based on ADCs also has obvious benefits for the environment by indirectly reducing nutrient outputs from farms (Cho, Hynes, Wood & Yoshida, 1994).

The investigation of potential feed ingredients for aquaculture species remains a priority as the global pressures on fish meal and fish oil continue to escalate (Tacon, 2003). This is especially so for carnivorous species which have traditionally relied on these two commodities to provide their basic dietary protein and energy requirements. This paper describes a series of experiments that determined the ADCs of a range of
diets and feed ingredients fed to sub-adult mulloway. Ingredients tested include fish meals, rendered animal by-product meals, oilseeds, legumes and cereals. To our knowledge, it is the first such paper detailing ADC's for this species.

2. MATERIALS AND METHODS

2.1 Overview

Three digestibility experiments were conducted. In each experiment a reference diet was used (i.e. formulated diet or commercial diet), and the digestibility of diets or ingredients were determined using indirect methods. Chromic oxide (Cr_2O_3) was used as the non-digestible marker. The inclusion content of individual test ingredients was varied depending on the design of each experiment, however, inclusion contents generally ranged from 10 to 50%, however, not all ingredients were tested at all levels. In all experiments, faecal material was collected by passive settlement from replicated groups of mulloway (n=3) that were housed in specially designed faecal collection tanks (Allan, Rowland, Parkinson, Stone & Jantrarotai, 1999). Fish from each experiment were acclimated to their respective experimental diets and laboratory conditions for different periods before faeces was collected, depending on the type of diet or ingredient being studied. New fish stocks were used for each experiment. A summary of each experiment is presented in Table 1.

2.2 Ingredients and diet preparation

The origin, measured nutrient and energy content of all test ingredients are presented in Table 2. Prior to inclusion in test feeds, all ingredients were ground through a hammer mill fitted with a 1.5mm screen (Raymond Laboratory Mill, Transfield Technologies, Rydalmere, NSW, Australia). The reference diet (or its constituent ingredients), test ingredients and marker were then combined on a dry weight basis and thoroughly mixed (Hobart Mixer; Troy Pty Ltd, Ohio, USA) before the addition of wet ingredients. Each mash was then formed into pellets of the appropriate size using a meat grinder fitted with a die plate (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia). Moist pellets were dried for 5 to 6 h (\approx 35° C) in a simple convection drier until moisture content was < 100 g kg-1 diet. Following preparation, all diets were stored frozen at < -15°C until required. The ingredient composition and measured nutrient or energy content of reference and experimental diets is presented in Table 3.

2.3 Fish stocks & handling protocols

The mulloway used in all experiments were progeny of brood-stock held at the NSW DPI Fisheries Port Stephens Fisheries Institute (PSFI). Prior to use in these experiments, mulloway were grown at low densities in large 10 kL holding tanks and fed once or twice daily on a commercial barramundi *Lates calcarifer* feed (Ridley Aqua-Feeds Pty. Ltd., Narangba, Qld, Australia).

Groups of mulloway were anaesthetised (10-25 mg L⁻¹ ethyl-p-aminobenzoate) and transferred from their holding facilities to the digestibility laboratory. A detailed description of this system is given in Allan, Rowland, Parkinson, Stone & Jantrarotai (1999), while the general procedures used to collect and store faeces from mulloway are described in Booth, Allan & Anderson (2005). Depending on the purpose of the experiment, some or all of the available digestibility tanks (190L cylindro-conical tanks) were stocked with mulloway of similar size and number (Table 1). After stocking at least 3 replicate tanks were randomly assigned to each dietary treatment. In all experiments, test diets were fed to excess (confirmed by the presence of uneaten feed in faecal collection tubes), over a period of approximately 3 h between 0830 and 1130 h each day. Once feeding had ceased, all tanks and collection tubes were cleaned and thoroughly rinsed before faecal matter was allowed to settle overnight (≈ 18 h). Faecal samples were

removed from settlement tubes the following morning prior to re-feeding. Daily faecal collections from individual tanks were pooled and kept frozen (< -15° C) until a sufficient quantity was obtained for chemical analyses. Afterwards, faecal samples were dried for 24h at room temperature in vacuum desiccators (70 mm Hg) using silica as a desiccant. Samples were finely ground (Waring, model 32 BL 80, New Hartford, Connecticut, USA) and re-dried (as described) prior to chemical analyses.

2.4 Chemical analyses

Chemical analysis of ingredients, diets and faecal material was done by various laboratories including the Food & Agricultural Laboratories of Australia Pty. Ltd. (FALA), Symbio – Alliance, Queensland Department of Primary Industries & Fisheries (QDPI&F), Industry and Investment NSW or Ecoteam Environmental Services. Analyses were conducted according to specific in-house methodologies or AOAC (1990)

2.5 *Calculation of digestibility coefficients*

Apparent digestibility coefficients (ADC) for reference and test diets were calculated according to equation 1:

ADC (%) =
$$100 \text{ x} [1 - (F/D \text{ x} D_{Cr}/F_{Cr})]$$
 Equation 1.

where F = % nutrient or gross energy in faeces; D = % nutrient or gross energy in diet; $D_{Cr} = \%$ chromic oxide in diet; $F_{Cr} = \%$ chromic oxide in faeces (Cho, Slinger & Bayley, 1982).

Apparent digestibility coefficients for ingredients were calculated according to equation 2:

 $ADC_{ING} (\%) = [(Nutr_{TD}*AD_{TD}) - (PRD*Nutr_{RD}*AD_{RD})] / [(P_{ING}*Nutr_{ING})]$ Equation 2.

where ADC_{ING} = apparent digestibility of nutrient or gross energy in the test ingredient; $Nutr_{TD}$ = the nutrient or gross energy concentration in test diet; AD_{TD} = the apparent digestibility of the nutrient or gross energy in the test diet; P_{RD} = proportional amount of reference diet; $Nutr_{RD}$ = the nutrient or gross energy concentration in the reference diet; AD_{RD} is the apparent digestibility of nutrient or gross energy in the reference diet; P_{ING} = proportional amount of test ingredient; $Nutr_{ING}$ is the nutrient or gross energy concentration in the test ingredient (Sugiura, Dong, Rathbone & Hardy, 1998).

3. **RESULTS AND DISCUSSION**

The calculated ingredient and nutrient composition of the reference and test diets used in each of the three experiments as well as their respective digestibility coefficients are presented in Tables 3-5. Apparent digestibility coefficients for ingredients are presented in Table 6.

As expected, the fish meal products obtained from sources in Peru and Ecuador were well digested and serve as benchmarks against which other potential ingredients for mulloway will be measured. Protein and energy digestibility coefficients for fish meal were similar at around 97%. Fat from fish meal was almost totally digested. These values reflect the digestibility coefficients determined for good quality fish meal in other carnivorous species including red sea bream (Yamamoto, Akimoto, Kishi, Unuma & Akiyama, 1998), snapper (Booth et al., 2005), red drum (Gaylord & Gatlin, 1996), gilthead sea bream (Lupatsch & Kissil, 1997) and rainbow trout (Cheng & Hardy, 2002). Low ash ovine and bovine meals were almost equivalent to fish meal in terms of protein and energy digestibility, however the fat digestibility of these products was lower. Allan et al. (unpublished data) determined the apparent digestibility coefficients for

the same products fed to Australian snapper at either 30 or 60% dietary inclusion level. Organic matter ADC's ranged from 84-100%, protein digestibility ranged from 83-95%, gross energy ADC's ranged from 82-96% and fat ADC's ranged from 85-96%. The apparent digestibility coefficients of the other rendered meals fed to mulloway were lower and more variable than the speciality meals, especially the batch of feather meal. The particularly low protein ADC of this product may indicate some rendering damage.

Pregelatinised wheat starch was better digested than extruded wheat. In addition, stepwise reductions in organic matter and gross energy ADC's of pregelatinised starch occurred as inclusion level increased. Booth et al. (2005; 2006) also reported a negative correlation between organic matter and gross energy ADC's for snapper fed pregelatinised wheat starch or extruded wheat. Such a clear correlation was not evident for mulloway fed increasing levels of extruded wheat, even though the dietary ADC's of diets containing extruded wheat declined in a similar manner to diets containing increasing levels of pregelatinised starch. The high inclusion levels of wheat and wheat starch tested in this study would not be used in commercial feeds for mulloway. However the data indicate that ADC's of carbohydrates are not additive, which has significant implications for feed formulation.

Sorghum protein was well digested but mulloway were unable to digest significant levels of organic matter or gross energy from this product. Protein from sorghum meal was reasonably well digested (ADC = 78%) by the omnivorous silver perch, but dry matter and gross energy digestibility were only 36.4 and 37.8%, respectively (Allan, Parkinson, Booth, Stone, Rowland, Frances & Warner-Smith, 2000). The digestibility of whole field peas presented a similar story. Both these products contain significant levels of carbohydrate in the form of fibre which may negatively impact on digestibility (McGoogan & Reigh, 1996). Mulloway were better at digesting the dehulled lupin meal than whole field peas. Significant improvements in the digestibility of dehulled legume seeds have been reported in silver perch (Booth, Allan, Frances & Parkinson, 2001). Solvent extracted soybean meal was more fully digested than either of the legumes we tested and will serve as a useful protein or energy source in diets for mulloway.

The digestibility coefficients determined in this study will be useful in formulating new commercial feeds as well as designing specific feeds for use in nutrition experiments with this species. These ADC's serve as a useful starting point for constructing a larger data base of ingredient digestibility coefficients for this species. This data base will become more important as the global pressures on fish meal and fish oil resources increase.

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Overview of digestibility experiments

	Experiment 1	Experiment 2	Experiment 3
Reference diet	formulated	formulated	commercial
Experiment tanks	10-40 24	30-50 24	50 24
Diet replication	3	3	3
Fish tank ⁻¹ Mean stock weight (g)	10 106	12 167	10 170
Weal stock weight (g)	100	107	170
Acclimation period (d)	14	7	7
Collection period (d)	9	6	9
Temperature (°C)	21-26	24-28	22-26
Dissolved oxygen (mg L ⁻¹)	5-6	5-6	5-6
Salinity (‰)	34-35	35-36	31-35
pH	7.5-7.8	7.4-7.8	7.5-7.9
$TAN (mg L^{-1})$	≤0.4	≤0.3	≤0.4

Experiment 1: 10, 20, 30 or 40% extruded wheat; 10, 20 or 30% pre-gelatinised wheat starch Experiment 2: 50% poultry offal meal, feather meal, meat meal, ovine meal or bovine meal; 30% blood meal Experiment 3: 50% Peruvian fish meal, Ecuador fish meal, dehulled lupin, soybean, sorghum, whole field peas

Measured nutrient and gross energy composition of ingredients used in each experiment (dry matter basis)

	Composition (g kg ⁻¹ or MJ kg ⁻¹)									
Ingredient	Experiment	Moisture ¹	Organic ² Matter	Ash	Crude protein	Fat	Gross energy			
Maize gluten ⁴	Exp 1	71.0	995.6	44	640.5	50.2	24 1			
Extruded wheat ³	Exp 1	118.6	971.8	28.2	172.6	47.0	19.2			
Fish meal - Chile ⁵	Exp. 1	108.3	827.0	173.0	754.2	89.1	20.7			
Pregel wheat starch ⁴	Exp. 1	68.1	997.1	2.9	5.4	1.4	17.6			
Blood meal ³	Exp. 2	53.0	971.5	28.5	978.9	10.6	24.3			
Bovine 70 ⁹	Exp. 2	83.0	966.2	33.8	848.4	146.1	24.9			
Extruded wheat ³	Exp. 2	116.0	970.6	29.4	171.9	55.4	18.9			
Feather meal ³	Exp. 2	81.0	978.2	21.8	868.3	105.5	24.6			
Fish meal ^{3,7}	Exp. 2	52.0	867.1	132.9	765.8	117.1	21.7			
Meat meal ^{3,6}	Exp. 2	44.0	599.4	400.6	507.3	87.9	14.6			
Ovine meal 60 ⁹	Exp. 2	62.0	820.9	179.1	736.7	107.7	21.2			
Ovine meal 70 ⁹	Exp. 2	66.0	899.4	100.6	815.8	109.2	22.8			
Poultry meal ³	Exp. 2	50.0	880.0	120.0	695.8	166.3	22.8			
Dehulled lupin meal ³	Exp. 3	98.0	971.0	29.0	429.4	70.0	20.2			
Fish meal - Ecuador ⁷	Exp. 3	62.0	864.0	136.0	770.0	102.0	21.1			
Fish meal – Peru ⁸	Exp. 3	81.0	822.0	178.0	727.5	87.0	19.7			
Pregel wheat starch ⁶	Exp. 3	91.0	996.0	4.0	na	na	16.8			
Sol. ext. soybean meal ³	Exp. 3	110.0	937.0	63.0	543.8	25.0	19.8			
Sorghum meal ¹⁰	Exp. 3	129.0	985.0	15.0	116.3	31.0	18.6			
Whole field-pea meal ³	Exp. 3	95.0	972.0	28.0	230.0	17.0	18.3			
Vit/min premix ¹¹	all exp's	158.7	662.0	337.8	148.0	71.0	13.8			

¹Moisture value provided to allow calculation of ingredient composition on as fed basis

² Organic matter by difference = (1000 - ash value); all tables ³ Ingredient sourced and provided by Ridley Aquafeed Pty. Ltd., Narangba, Qld, Australia

⁴ Penford Australia Ltd., Lane Cove, NSW, Australia

⁵ Imported steam dried fish meal, Pesquera Itata, Chile, South America; batch 2

⁶ Australian Meat Holdings (AMH) Pty. Ltd., Dinmore, Qld, Australia

⁷ Imported steam dried fish meal with antioxidant, Empresa Pesquera Polar, Ecuador

⁸ Imported steam dried fish meal, high quality, Grupo Sindicato, Pesquero Del Perus, FEMAS, SA

⁹ Bakels Edible Oils (N.Z.) Ltd., Mt Maunganui, New Zealand

¹⁰ NSW DPI Fisheries PSFI feed stock

¹¹ NSW DPI Fisheries formulation; prepared by CCD Animal Health & Nutrition, Toowoomba, Qld, Australia

Ingredient and measured nutrient composition of diets used in experiment 1 (g kg⁻¹ or MJ kg⁻¹ dry matter basis)

	Ref	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Ingredient composition	1							
Fish meal	800.0	720.0	640.0	560.0	720.0	640.0	560.0	480.0
Maize gluten	110.0	99.0	88.0	77.0	99.0	88.0	77.0	66.0
Fish oil	70.0	63.0	56.0	49.0	63.0	56.0	49.0	42.0
Vit/min premix	15.0	13.5	12.0	10.5	13.5	12.0	10.5	9.0
Cr^2O_3	5.0	4.5	4.0	3.5	4.5	4.0	3.5	3.0
Pregel wheat starch	-	100.0	200.0	300.0				
Extruded wheat	-	-	-	-	100.0	200.0	300.0	400.0
Nutrient composition								
Organic matter	852.7	865.7	882.1	893.2	864.9	874.7	885.5	898.8
Ash	147.3	134.3	117.9	106.8	135.1	125.3	114.5	101.2
Crude protein	670.7	607.8	540.0	472.3	623.5	578.5	531.2	477.8
Total fat	147.4	134.1	100.2	76.2	137.0	127.1	117.4	104.6
Gross energy	22.6	22.2	21.5	21.0	22.1	21.8	21.4	21.2
% Apparent digestibilit	y of diet (n	nean±SEM)					
Organic matter	88.4±0.4	89.3±0.5	86.1±0.6	80.0±1.4	83.5±1.1	79.9±2.1	72.4±0.8	66.9±1.2
Crude protein	92.0±0.0	92.1±0.5	91.2±0.2	89.1±0.4	92.1±0.7	92.1±0.1	91.3±0.2	91.4±0.6
Total fat	98.6±0.1	98.5±0.3	97.9±0.3	95.0±0.6	98.6±0.1	98.3±0.2	98.1±0.1	97.7±0.3
Gross energy	92.2±0.2	91.6±0.7	88.9±1.0	83.6±0.9	88.0 ± 0.7	84.4±1.3	79.1±0.5	74.3±0.9

¹ See Table 2 for key to ingredients

Ingredient and measured nutrient composition of diets used in experiment 2 (g kg⁻¹ or MJ kg⁻¹ dry matter basis)

	Ref	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Ingredient composition ¹								
Fish meal	490.0	245.0	245.0	245.0	343.0	245.0	245.0	245.0
Extruded wheat	480.0	240.0	240.0	240.0	336.0	240.0	240.0	240.0
Vit/min premix	20.0	10.0	10.0	10.0	14.0	10.0	10.0	10.0
Cr^2O_3	10.0	5.0	5.0	5.0	7.0	5.0	5.0	5.0
Poultry meal	-	500.0	-	-	-	-	-	-
Feather meal	-	-	500.0	-	-	-	-	-
Meat meal	-	-	-	500.0	-	-	-	-
Blood meal	-	-	-	-	300.0	-	-	-
Ovine meal 60	-	-	-	-	-	500.0	-	-
Ovine meal 70	-	-	-	-	-	-	500.0	-
Bovine meal 70	-	-	-	-	-	-	-	500.0
Nutrient composition								
Organic matter	902.3	891.6	940.7	753.1	924.1	875.5	904.9	926.5
Ash	97.7	108.4	59.3	246.9	75.9	124.5	95.1	73.5
Crude protein	466.0	570.6	664.2	489.7	613.3	601.0	646.9	657.0
Total fat	87.0	128.8	100.8	85.4	57.2	94.9	99.4	133.2
Gross energy	20.1	21.2	22.5	17.7	20.9	20.8	21.6	22.2
% Apparent digestibility	of diet (mear	n±SEM)						
Organic matter	55.0±2.6	66.1±0.5	58.9±1.1	67.1±0.9	62.5±1.4	78.5±1.7	7 75.8±3.0) 76.3±3.0
Crude protein	90.8±0.5	81.8±1.4	69.1±3.3	8 87.2±0.2	2 90.6±0.5	92.5±0.8	8 92.9±1.1	93.7±0.9
Total fat	89.1±2.2	88.1±1.2	2 73.4±1.2	2 83.3±1.2	2 81.8±0.7	83.7±1.8	8 86.5±5.3	8 81.1±2.5
Gross energy	62.9±1.9	71.1±0.7	62.0±1.3	3 71.9±0.8	8 68.6±1.2	81.4±1.5	5 79.8±2.9	9 79.3±2.7

¹ See Table 2 for key to ingredients

Ingredient and measured nutrient composition of diets used in experiment 3 (dry matter basis)

	Ref	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Ingredient composition ¹								
Commercial feed [†]	960.0	480.0	480.0	480.0	480.0	480.0	480.0	-
Pregel wheat starch	30.0	15.0	15.0	15.0	15.0	15.0	15.0	10.0
Cr^2O_3	10.0	5.0	5.0	5.0	5.0	5.0	5.0	10.0
Fish meal – Ecuador	-	500.0	-	-	-	-	-	-
Fish meal – Peru	-	-	500.0	-	-	-	-	-
Dehulled lupin meal	-	-	-	500.0	-	-	-	-
Whole field-pea meal	-	-	-	-	500.0	-	-	-
Sorghum meal	-	-	-	-	-	500.0	-	-
Sol. ext. soybean meal	-	-	-	-	-	-	500.0	-
Commercial feed [‡]	-	-	-	-	-	-	-	980.0
Nutrient composition (g	kg ⁻¹ or MJ	kg ⁻¹)						
Organic matter	895.0	880.0	853.0	931.0	931.0	939.0	914.0	910.0
Ash	105.0	120.0	147.0	69.0	69.0	61.0	86.0	90.0
Crude protein	490.6	628.8	609.4	463.08	362.5	305.0	518.1	455.0
Total fat	123.0	108.0	105.0	92.0	69.0	77.0	72.0	187.0
Gross energy	21.5	20.9	20.6	21.1	20.2	19.6	20.7	22.2
% Apparent digestibility	of diet (me	ean±SEM)						
Organic matter	78.5±1.2	87.2±0.4	86.5±0.3	66.0±0.7	55.2±1.0	47	72.5±1.0	78.7±2.2
Crude protein	82.2±1.2	91.1±0.3	90.7±0.3	89.9±0.4	84.6±0.6	84	88.0±0.8	87.7±1.8
Total fat	97.3±0.4	98.5±0.2	98.2±0.6	92.9±0.5	94.5±0.7	89	95.3±0.4	97.7±0.1
Gross energy	82.2±1.3	89.9±0.4	89.5±0.4	74.6±0.7	62.6±1.3	55	78.5±0.8	83.9±1.7

¹ See Table 2 for key to ingredients

[†]Commercial barramundi feed 50:12 (Ridley Aquafeed Pty Ltd., Narangba, Qld, Australia) [‡]Commercial barramundi feed 45:20 (Ridley Aquafeed Pty Ltd., Narangba, Qld, Australia)

N.B. Only one replicate value was available for calculation of diet ADC for sorghum

Percent apparent digestibility coefficients (ADC) of selected ingredients fed to juvenile mulloway

		А	pparent digesti	bility coefficie	ent %	
Ingredient	Exp.	Inclusion %	Organic matter	Crude protein	Gross energy	Fat
Fish meals						
Fish meal - Ecuador	Exp. 3	50	96.3	96.8	97.7	99.9
Fish meal – Peru	Exp. 3	50	96.8	96.4	97.4	99.5
Rendered by-product me	als					
Meat meal	Exp. 2	50	85.3	83.9	84.3	77.6
Poultry meal	Exp. 2	50	77.5	75.8	78.3	87.6
Feather meal	Exp. 2	50	62.5	57.4	61.3	60.5
Blood meal	Exp. 2	30	78.8	90.4	79.6	-
Ovine meal 60	Exp. 2	50	104.3	93.6	97.7	79.3
Ovine meal 70	Exp. 2	50	96.7	94.1	95.8	84.4
Bovine 70	Exp. 2	50	96.2	95.3	92.5	76.3
Cereals						
Extruded wheat	Exp. 1	10	44.8	88.5	43.6	98.6
	Exp. 1	20	50.1	90.4	47.7	94.5
	Exp. 1	30	39.6	85.1	43.2	94.4
	Exp. 1	40	38.6	88.0	42.8	93.5
Pregel wheat starch	Exp. 1	10	96.2	92.1	84.7	-
e	Exp. 1	20	78.2	-	72.0	-
	Exp. 1	30	63.2	-	57.9	-
Sorghum meal	Exp. 3	50	18.8	91.6	23.6	56.1
Legumes / oilseeds						
Sol. ext. sovbean meal	Exp. 3	50	68.2	93.2	74.5	85.5
Dehulled lupin meal	Exp. 3	50	54.9	98.7	66.5	85.2
Whole field-pea meal	Exp. 3	50	33.8	89.7	39.5	74 2
male neta peu meur	LAP. J	50	55.0	07.1	57.5	/ 1.2

4.3 Response of juvenile mulloway *Argyrosomus japonicus* to an intra-peritoneal injection of D-glucose

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1. INTRODUCTION

Increasing effort is being made to use more plant ingredients in aquatic animal feeds. This is because plant based ingredients may provide a cheaper source of digestible protein or energy (i.e. as opposed to lipid or protein based ingredients) and secondly because the carbohydrate fraction of feed ingredients imparts enhanced physical qualities to extruded feeds. However, increasing the use of plant ingredients increases the level of dietary carbohydrate (CHO) which has implications for the nutrition of most fish species (Stone, 2003). Plant CHO's may be classified as either reserve polysaccharides or structural polysaccharides. For example, the polysaccharide starch is a major energy reserve in most grains and legumes, and one of the principal components of wheat (~80%) and field peas (~40%). Most vertebrates have some ability to digest and utilise the energy from starch after it is reduced to monomers of α -glucose. Glucose serves as the primary energy source in mammalian metabolism, however its role in fish metabolism is not as well understood (Moon, 2001; Hemre, Mommsen & Krogdahl, 2002; Stone, 2003). The major structural polysaccharide in plants is generally cellulose. Although cellulose is also made of a monomer of glucose (i.e. β -glucose), the structural properties of cellulose are very different from starch. The enzymes that digest starch by hydrolysing the α -bonds are unable to hydrolyse the β -linkages, making it essentially indigestible by vertebrates (Campbell, 1996).

Preliminary investigations of CHO utilisation in fish and other species are often undertaken using a simple glucose tolerance test (Moon, 2001; Stone, Allan & Anderson, 2003a). A carbohydrate such as glucose is introduced orally or injected into the peritoneum and then the uptake and clearance of the carbohydrate from the blood is monitored over time. This has been done on a variety of established aquaculture species including silver perch (Stone et al., 2003a), barramundi (Anderson, 2002), tilapia (Shiau & Chuang, 1995) and red sea bream (Koshio, 2002; Booth, Anderson & Allan, 2006). In general, fish of a low trophic level tend to be more efficient in the uptake and clearance of glucose from the blood stream compared to carnivorous species (Furuichi & Yone, 1981; Garcia-Riera & Hemre, 1996; Peres et al., 1999). However, most fish species exhibit a prolonged state of hyperglycaemia when subjected to acute loads of glucose and in a clinical sense, are considered to exhibit impaired glucose tolerance (Wilson, 1994; Moon, 2001; Stone, 2003a).

Mulloway (*Argyrosomus japonicus* previously described as *A. hololepidotus*) are a large, estuarine sciaenid of recreational and commercial importance in Australia and South Africa (Fiedler & Bardsley, 1999). They are widely distributed in the temperate waters of the African southeast coast, the entire southern seaboard of Australia, the northern Indian Ocean off Pakistan, the northwest coast of India as well as the Northern Pacific from Hong Kong, along the Chinese coast, up to Japan and Korea (Griffiths & Heemstra, 1995). In Australia, mulloway grown for commercial purposes are reared in sea cages and fed on proprietary aqua-feeds used for a variety of species including Atlantic salmon, barramundi and yellowtail kingfish. They are considered carnivorous by nature, but little is known about their nutritional

requirements or their ability to digest and utilise the protein, lipid or carbohydrate components of different diets or feed ingredients.

This study details a preliminary investigation of CHO utilisation in juvenile mulloway by measuring the uptake and clearance of an intra-peritoneal injection of 1 g D-glucose kg^{-1} body weight (BW) from the blood plasma.

2. MATERIALS AND METHODS

2.1 Fish

Approximately 120 juvenile mulloway were obtained from a commercial fish hatchery (O'Donohue Filter Sand & Gravel Pty. Ltd., Millers Forest, NSW, Australia) and transported to NSW DPI Port Stephens Fisheries Institute (PSFI). Prior to use in the experiment the fish were held in a 10 kL holding tank located inside a large plastic covered shade-house. The holding tank was connected to a saltwater recirculating system and the water temperature was maintained at $21 \pm 2^{\circ}$ C. Fish were fed a commercial sinking pellet twice daily (Ridley Aquafeed Pty. Ltd., Narangba, Queensland, Australia). One week before the experiment mulloway (weight range 86-288 g) were anesthetised (15-20 mg L⁻¹ ethyl-p-aminobenzoate) and graded into four size classes. Each group (≈ 26 fish) was placed in a separate 100 L circular cage (perforated oyster mesh) that had been suspended and secured in the holding tank. Each group was then randomly selected to conduct one of four repetitions of the glucose tolerance trial. All fish in this experiment were injected, handled or sampled only once. Afterwards they were returned to holding tanks to recover.

Injection & sampling procedures

A concentrated solution of D-glucose was prepared by dissolving 50 g anhydrous analytical grade D-glucose powder (Ajax Finechem, Seven Hills, NSW, Australia) in 100 mL of sterilised distilled water (0.5 g mL⁻¹ standard solution). In addition, two control treatments were employed to confirm that neither handling nor injection procedures unduly influenced plasma glucose concentrations (Stone et al., 2003a; Booth et al., 2006). Fish subjected to the sham control were injected with a sterile saline solution (0.9% sodium chloride; AstraZeneca), while fish subjected to the handling control were exposed to exactly the same experimental procedures but were not injected. The volume of glucose or saline solution injected into each fish varied slightly according to fish weight.

Each group of mulloway was fasted for approximately 72 h prior to beginning a trial. On each occasion, fish were lightly sedated (20 mg L-1 ethyl- ρ -aminobenzoate) before 3 fish were randomly selected and immediately sampled to establish resting plasma glucose concentrations (i.e. 0 hour). Following this procedure individual fish were weighed and then randomly allocated to one of the 3 experimental treatments (glucose, sham or handling). The time of treatment was recorded and each fish was placed in a separate holding tank until required (i.e. 1, 3, 6, 12, 24, 48 or 72 hours after treatment; 21 samples). Water quality within tanks during each trial was maintained at a temperature of $21 \pm 1^{\circ}$ C, pH between 7.7 ± 0.2 and salinity of $29 \pm 3\%$. Dissolved oxygen concentrations were maintained at acceptable levels (> 5mg L⁻¹) by placing an air stone infuser in each cage. At the appropriate times individual fish were captured without anaesthetic and restrained in a soft foam block such that the ventral surface of the fish was exposed. Approximately 2 mL of blood was then withdrawn from the caudal vein using a 23 gauge x 1.25 mm hypodermic needle and a 3 mL syringe (Becton-Dickinson B-D, Singapore). Blood samples were collected within 90 seconds of capture to prevent stress mediated glucose responses (Stone et al., 2003a). To prevent haemolysis, needle tips were removed before whole blood samples were transferred into specialised 2 mL collection tubes prepared to prevent coagulation and halt glycolysis (VACUETTE

Greiner Bio-one FE; Sodium Flouride / EDTA K3). Labelled samples were refrigerated and immediately transferred to a NATA accredited pathology laboratory for analysis of plasma glucose.

2.2 Chemical analysis

Plasma glucose was determined using an enzymatic reference method that used hexokinase to convert Dglucose to NADH. The concentration of NADH was determined by measuring its absorbance at 340 nm (COBAS INTEGRA 700; Hunter Area Pathology Service, Newcastle, NSW, Australia).

2.3 Statistical analysis

Previous GTT experiments have been analysed using two-way multifactor ANOVA which have typically demonstrated a strong interaction between treatment factors (glucose, sham or handling) and time of measurement due to the dramatic elevation in the glucose levels of fish injected with D-glucose compared to fish injected with a saline solution or subjected to a handling stress (Stone, Allan & Anderson, 2003a; Booth et al., 2006). For this reason the data obtained from this study are compared using an unreplicated randomised complete block design with treatment (glucose, sham or handling) and sampling time (0, 1, 3, 6, 12, 24, 48 or 72 hours) as the two fixed factors of main interest and "trial group" (group 1, 2, 3 or 4) as a random blocking factor. Block by treatment interactions were included in the model (Newman, Bergelson & Grafen, 1997; Quinn & Keogh, 2002). The effect of the procedural controls on glucose response was minimal compared to the effect of injecting 1 g D-glucose kg⁻¹ BW, therefore only the effect of time on each treatment was considered in more detail. As in the previous statistical analysis, trial group was used as the blocking factor. Separation of treatment means was done using the *Duncans* procedure with alpha set at 0.05. Statistical analysis was performed using NCSS (NCSS, Kaysville, Utah; release 2006) and Statgraphics Plus V4 (Manugistics, USA).

3. **RESULTS**

The injection of 1 g D-glucose kg⁻¹ BW into the peritoneal cavity of mulloway resulted in the immediate elevation of plasma glucose levels (Figure 1). Factorial analysis of the data indicated there were highly significant effects of treatment type, time of sampling and the interaction of treatment and time on the plasma glucose levels of juvenile mulloway (Table 1). The level peaked at 21.93 \pm 0.79 mM approximately six hours after injection and remained elevated for up to 48 hours (Figure 1). SOne way ANOVA indicated time of sampling significantly affected the concentration of glucose in the plasma of fish injected with D-glucose (P<0.0001). There was a statistical similarity in the concentration of glucose in fish sampled at 0 hours with those sampled 24, 48 or 72 hours post injection (Table 2). Time of sampling also affected the plasma glucose levels of mulloway subjected to the saline procedural control (P<0.0116), with fish sampled 1 hour post injection recording a moderate but significant variation in plasma glucose concentrations over time.

4. DISCUSSION

The ability to absorb and rapidly regulate plasma glucose to basal circulating levels after either an injected or fed dose of highly available CHO (such as glucose) is used as a relative measure of CHO tolerance (Moon, 2001). Accordingly, the assimilation and prolonged hyperglycaemia exhibited by juvenile mulloway indicates impaired glucose homeostasis when glucose is administered via the intra-peritoneal cavity.

The general method of carbohydrate digestion is hydrolysis of complex carbohydrates extracellularly in the stomach, intestine, and caeca, with membrane-linked hydrolysis in the anterior intestine and caeca by a variety of carbohydrases. The products of this hydrolysis are simple carbohydrates (polysaccharides and monosaccharides). It is known that in mammals the transportation of monosaccharides from the lumen of the small intestine is by an active transport mechanism in the brush border. However it is unclear whether this is also true in fish (Rust, 2002). Differences in carbohydrate digestibility amongst species are in part attributable to the different amounts and types of carbohydrases. The relative utilization of dietary carbohydrate varies and appears to be related to the complexity of the carbohydrate. In general, cooked starch and dextrin are better utilised by fish than simple sugars. The prolonged hyperglycaemia observed in fish following glucose tolerance tests and their relative inability to utilise simple sugars has been attributed to a few factors including low hexokinase activity and a lack of an inducible glucokinase enzyme; glucose not being as potent a stimulus for insulin release as many amino acids; the possible inhibition of insulin by somatostatins released in response to high blood glucose levels; and the relatively low number of insulin receptors in fish compared to in mammals (Wilson, 1994).

The glucose response in mulloway is similar to that reported for other carnivorous fish such as gilthead seabream and European seabass (Peres, Goncalves & Oliva-Teles, 1999) and turbot *Scophthalmus maximus* (Garcia-Riera & Hemre, 1996), challenged with an intra-peritoneal injection of glucose, but was not as rapid as the uptake or clearance of glucose in the plasma of the more omnivorous silver perch (Stone et al., 2003a). The majority of evidence for poor glucose removal from the plasma compartment in fish points to mechanisms involving either a lack of peripheral white muscle glucose transporters sensitive to insulin or other rate limiting steps in glucose metabolism (Wright, O'Hali, Yang, Han & Bonen, 1998; Moon, 2001; Hemre et al., 2002; Gisbert, Sainz & Hung, 2003; Stone, 2003). The fate of glucose assimilated by mulloway in the present study was not tested, however several pathways for clearance of excess glucose exist in fish including glycosuria (Deng, Refstie & Hung, 2001) and excretion across the gills (Stone, 2003a).

Previous research has shown that some carnivorous species that have not performed well in GTT's but that are fed a diet low in CHO for a prolonged period can still exhibit good growth rates, increased glucose tolerance, efficient adaptation of hepatic carbohydrate-metabolizing enzymes and demonstrate a protein-sparing effect (Shimeno, Hosokawa & Takeda, 1979). Carnivorous yellowtail grew well on diets with up to 20% carbohydrate (Shimeno et al., 1979), and red drum tolerated up to 35% carbohydrate in their diet without evidence of detrimental effects on growth (Gaitlin, 2002). Growth trials substituting wheat starch at different inclusion levels could indicate if carbohydrate can have a protein-sparing effect in mulloway. Ellis and Reigh (1991) found that red drum appeared to have a limited ability to utilise dietary carbohydrate as an energy source. Dietary energy levels and the carbohydrate content were inversely related to weight gain, feed efficiency, apparent net protein retention and apparent net energy retention. A greater protein sparing effect was observed from diets incorporating high levels of dietary lipids.

The literature concerning GTT's has emphasized the confounding effects of stress induced responses on circulating glucose concentrations such as handling, injection, and repeated disturbance. This issue must be adequately addressed during GTT's as stress has been found to elevate plasma glucose levels in numerous studies (Robertson et al., 1987; Robertson et al., 1988). To confirm that handling and injection procedures did not significantly influence plasma glucose concentrations two procedural controls were included in our experiment. Although there was a slight increase in glucose concentrations above initial levels for both these controls, the magnitude of the rise was relatively small. The rapid initial glucose rise that occurs after exposure to stressors in fish is due mainly to increased catecholamine secretion, whilst the sustained hypoglycaemia is thought to be attributable to cortisol (Robertson et al., 1988).

The evidence collected from a simple glucose tolerance test indicates that juvenile mulloway have impaired glucose homeostasis when glucose is administered via the intra-peritoneal cavity. Comparative studies investigating the assimilation of simple and complex carbohydrates digested and absorbed via the lumen would complement this study and increase our knowledge of CHO utilisation in this species.

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Results of factorial ANOVA on two fixed factors (treatment or time) and a random blocking factor (trial number).

Source Term	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level
A: Treatment	2	1473.254	736.627	163.16	0.000006*
B: Trial	3	51.43542	17.14514		
AB	6	27.08771	4.514618		
C: Time	7	666.9429	95.27756	15.45	0.000001*
AC	14	1138.578	81.32698	16.53	0.000000*
BC	21	129.5079	6.167044		
ABC	42	206.674	4.920808		
S	0	-4.915968E-13			
tal (Adjusted)	95	3693.479			

TABLE 2

Mean \pm sem of plasma glucose concentrations (mM) in juvenile mulloway sampled over a 72 hour period following injection or handling procedures.

Glucose	Sham	Handled	
2.40 ± 0.54^{a}	2.22 ± 0.20^{ab}	2.02 ± 0.50	
2.40 ± 0.34	2.33 ± 0.29	2.93 ± 0.39	
17 12 \pm 2 72 ^c	$4.03 \pm 0.65^{\circ}$	2.00 ± 0.67	
17.13 ± 3.72	4.03 ± 0.03	3.00 ± 0.07	
10 55 $\pm 2.61^{\circ}$	3.55 ± 0.00^{bc}	3.02 ± 1.27	
19.33 ± 2.01	5.55 ± 0.99	5.25 ± 1.27	
21.02 ± 0.70 ^c	1.05 ± 0.25 ^a	1 55 ± 0.46	
21.93 ± 0.79	1.95 ± 0.35	1.33 ± 0.40	
11 30 + 2 3 ^b	1.73 + 0.21 ^a	1.85 ± 0.17	
7.68 ± 1.54^{ab}	1.75 ± 0.21 2 38 + 0 56 ^{ab}	1.05 ± 0.17 1.75 ± 0.32	
7.00 ± 1.34	2.38 ± 0.30	1.75 ± 0.32	
3 40 + 0 38 ^a	1 88 + 0 34 ^a	1.95 + 0.21	
2.55 ± 0.36^{a}	2.55 ± 0.17^{ab}	2.30 ± 0.37	
	$Glucose$ 2.40 ± 0.54^{a} 17.13 ± 3.72^{c} 19.55 ± 2.61^{c} 21.93 ± 0.79^{c} 11.30 ± 2.3^{b} 7.68 ± 1.54^{ab} 3.40 ± 0.38^{a} 2.55 ± 0.36^{a}	$\begin{array}{c c} \textbf{Glucose} & \textbf{Sham} \\ \hline 2.40 \pm 0.54^{a} & 2.33 \pm 0.29^{ab} \\ 17.13 \pm 3.72^{c} & 4.03 \pm 0.65^{c} \\ 19.55 \pm 2.61^{c} & 3.55 \pm 0.99^{bc} \\ 21.93 \pm 0.79^{c} & 1.95 \pm 0.35^{a} \\ 11.30 \pm 2.3^{b} & 1.73 \pm 0.21^{a} \\ 7.68 \pm 1.54^{ab} & 2.38 \pm 0.56^{ab} \\ 3.40 \pm 0.38^{a} & 1.88 \pm 0.34^{a} \\ 2.55 \pm 0.36^{a} & 2.55 \pm 0.17^{ab} \\ \end{array}$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

Different superscript letters in each column indicate means were significantly different.



FIGURE 1

Effect of glucose injection, sham injection or handling on plasma glucose concentrations in juvenile mulloway.

4.4 The effect of stocking density and repeated handling on the growth of juvenile mulloway, *Argyrosomus japonicus* (Temminck & Schlegel, 1843)

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ABSTRACT

The effect of stocking density on the growth of mulloway, *Argyrosomus japonicus*, was tested with 17 g fish stocked at 4.08, 8.16 or 16.32 kg m⁻³ in 50 L aquaria. Weight checks were carried out every two weeks to track performance. Each density treatment was also compared to a non-handled control group to establish if handling during weight checks influenced the growth of mulloway. Mulloway performed poorly at the lowest density and, under the current experiment conditions, growth did not appear to be negatively affected by regular handling.

1. INTRODUCTION

Mulloway, *Argyrosomus japonicus*, are a commercially and recreationally important sciaenid species in Australia and efforts in recent years have focused on improving production techniques for wild-stock enhancement and aquaculture of the species (Fielder & Bardsley, 1999; Fielder et al., 1999). As a new aquaculture species relatively little is known of the effects that various environmental factors have on the growth of mulloway. Stocking density is one of the most important biotic factors influencing growth and feed intake of fish in culture (Kestemont & Baras, 2001) directly modifying feeding behaviour (Boujard et al., 2002), social interactions (Barcellos et al., 1999), water quality (Ellis et al., 2002), and has also been shown to influence sexual dimorphism (Davis et al., 2002). Stocked densities of 15 kg m⁻³ at harvest have been achieved for mulloway (Quartararo, 1996) however the relationship between stocking density and growth of mulloway is currently unknown.

The primary objective of this study was to identify the effects of stocking density on the growth of juvenile mulloway as evidenced by survival, body weight and length, condition factor, size heterogeneity and feeding efficiency. This information will be of use in determining appropriate stocking densities of mulloway for both future growth studies and aquaculture.

During growth studies on fish it is common practice to track performance (growth) over time by sampling periodically and measuring some physical parameter, e.g. weight, length, etc. Anaesthetics are commonly used to minimize the stress response when handling fish; however, anesthesia can itself produce a stress response (Ortuno et al.., 2002a, b) and can also have a negative effect on growth (Hoskonen & Pirhonen, 2006). Each stocking density treatment was therefore also compared to a non-handled control group to identify if the growth of mulloway is compromised from routine handling during regular weight checks.

2. MATERIALS AND METHODS

The effect of density on the growth of mulloway was tested over 37 days using 17 g fish (± 3.5 g), 4 month old, F2 juveniles of broodstock held at the New South Wales Department of Primary Industries, Port Stephens Fisheries Institute (PSFI). Fish were sedated with using 20 mg L⁻¹ benzocaine (ethyl <u>p</u>-aminobenzoate) and stocked into 50 L aquaria at one of three stocking densities: 4.08, 8.16 or 16.32 kg m⁻¹

³ (12, 24 or 48 fish aquaria⁻¹), nominally low (LD), medium (MD) and high (HD) densities respectively. There were four replicate aquaria for each density treatment. The control (non-handled) group consisted of an additional four replicate aquaria for each of the three stocking densities. Once stocked, the control fish were not handled until the completion of the experiment. In this experiment the combined effects of anaesthesia and handling cannot be separated and therefore the terms 'handling' or 'handled' are used to denote both.

The experiment system consisted of 24 x 50 L replicate acrylic aquaria integrated via a semi-recirculating bio-filtration unit. A moderate flow-through rate allowed twice daily renewal of water to the system. Flow to each aquarium was approximately 2 L min⁻¹ ensuring similar water quality between all treatment aquaria. Ranges and means (\pm SD) for water quality parameters were: temperature ($^{\circ}$ C) 19.6 – 22.5, 20.8 (0.9); NH₄⁺ (mg/L) 0.1 – 0.8, 0.4 (0.1); DO (mg/L) 5.3 – 7.2, 6.1 (0.3); pH 7.8 – 8.3, 8.0 (0.1); salinity (ppt) 26.0 – 32.3, 29.4 (1.4). Black plastic sheets were placed between each aquarium and across the front to minimize disturbance. All aquaria were exposed to 12L:12D photoperiod using fluorescent lighting (<1 μ E m⁻² s⁻¹ at aquaria surface).

Analysis of variance of initial weights ($F_{2,21} = 3.35$; P > 0.05) and initial CV ($F_{2,21} = 0.76$; P > 0.1) demonstrated no significant difference between treatments. An additional 100 individuals were also measured for weight and total length (L_T) for initial condition factor (K) comparison. Refer to Table 1 for summary of initial data.

Weight checks were carried out every two weeks on the handled treatment group. To ensure that handling protocols during weight checks remained consistent between all density treatments, fish in the highest density were sampled first and the exposure time to handling and benzocaine per aquarium noted. This time (approx. 15 min.) was then applied to the remaining densities and also to subsequent weight checks.

Fish were fed by hand twice daily (08:30 & 15:00) to apparent satiation with a commercial barramundi (*Lates calcarifer*) diet (Ridley AquaFeed Pty. Ltd., Narangba, Qld. Australia; reported nutrient composition: 50% crude protein, 12% crude fat, 2.5% fibre, 18 MJ kg⁻¹ gross energy) which was reground and repelleted (3mm) to sink.

Aquaria were inspected daily and any mortalities were replaced with similar size fish in order to maintain treatment densities. Replacement fish were fin clipped (left pectoral) for ease of identification and were not used in the final analyses; all data were derived from the tank means of the remaining original fish. Faeces and feed debris were siphoned from tanks daily. Shoaling and feeding behaviour and responses to routine aquaria maintenance were observed daily; however, these were not quantified.

Co-efficient of variation (CV) of weight (%), Condition factor (K) and feeding efficiency (FE) were calculated as:

 $CV = s.\overline{x}^{-1} x 100$ $K = [W / L_T^{-3}] x 100$

where,

W = wet weight (g) and L_T = total length (cm).

FE = wet weight gain (g) / total feed intake (g).

A 2-way ANOVA was used to determine density and handling effects on the dependent variables: survival (%), final weight (W_f), final length (L_f), FE, CV and K. Cochran's C test was used to test homogeneity of variances. Tukey-Kramer test was used for *a-posteriori* multiple comparison of means on significant terms. Probability of Type I error was set at $\alpha = 0.05$ for all analyses.

3. **RESULTS**

There was no significant interaction or handling main effect between densities for all variables (survival (%), W_f , L_f , K, CV, FE) (Table 2). The handling term was therefore removed and all subsequent analyses performed as a single factor ANOVA on pooled data.

Mean individual weights were significantly different between density treatments from the first weight check two weeks after initial stocking ($F_{2,9} = 6.35$; P < 0.02) (Figure 1). At week two MD fish were larger than LD fish but not significantly different from the HD fish. By week four both the MD and HD fish were larger than the LD fish ($F_{2,9} = 8.05$; P < 0.01) (Figure 1). The effect of stocking density was also significant on final weight ($F_{2,21} = 12.35$; P < 0.001) and final length ($F_{2,21} = 20.48$; P < 0.001) with MD and HD fish larger than LD fish (Table 1). Stocking densities (\pm SD; n = 8) at the conclusion of the experiment were 5.7(0.5), 13.8(1.1) and 26.4(1.9) kg m⁻³ for LD, MD and HD respectively.

Total overall survival was 88%. There was a trend for greater survival with decreasing density; however, this was not statistically significant ($F_{2,21} = 1.87$; P > 0.1) (Table 1).

Final FE was significantly poorer for the LD treatment than MD and HD treatments (Table 1). CV increased from initial stocking (Table 1); however there was no significant difference between final density treatments ($\underline{F}_{2,21} = 1.07$; P > 0.2; Table 1).

Initial and final condition co-efficients were similar (Table 1). Stocking density did not have a significant effect on final <u>K</u> ($\underline{F}_{2,21} = 0.72$; P > 0.5). Heterogeneity of variances could not be removed from final <u>K</u> data; however, ANOVA was still performed. The result is valid as heterogeneous data increases the chance of Type I error (Underwood, 1997) and, in this case, there were no significant differences.

There was no agonistic behaviour observed during feedings or at other times in any of the aquaria. LD fish appeared to be quite timid for the first two weeks; often staying in the back corner of aquaria huddled together and taking longer to approach food. In contrast, MD and HD fish were evenly dispersed throughout aquaria. Fish did not appear to be disturbed by daily siphoning of aquaria. Lights switching on and off startled the fish causing them to swim rapidly for several seconds and collide with the aquaria surfaces; however, normal behaviour appeared to resume quite quickly after each event.

4. **DISCUSSION**

The results indicate an appropriate initial (~17g fish) lower stocking threshold for mulloway of above 4.08 kg m⁻³ while growth between MD and HD were similar suggesting an initial stocking density in excess of 16.32 kg m⁻³ may be achievable. While the direct extrapolation of MD or HD stocking densities used in this experiment to commercial scale culture or different size classes of mulloway may not be appropriate it is important to note that this study demonstrated the significant negative effect of low stocking density on the growth of mulloway after only two weeks.

Under the current experiment conditions mulloway were not negatively affected by regular handling. Negative growth responses to anaesthesia may be anaesthetic specific (e.g. Hoskonen & Pirhonen, 2006) and in this case mulloway appear to be able to tolerate regular weight checks using benzocaine. It should

be noted however that exposure to a repeated stressor can potentially reduce the ability of fish to respond to an additional acute stressor (Barton, 2002). It is unclear to what extent, if any, that the daily switching on and off of lights (repeated stressor) masked the additional effect of handling (acute stressor) on the growth of mulloway in this experiment. Growth of MD mulloway in this experiment was however comparable to those of juvenile mulloway in intensive culture using 10000 L tanks (~0.35g day⁻¹) (Booth, Allan & Losordo, unpublished Data).

LD fish fed erratically; reluctant to feed when food was introduced into the aquaria then darting over to pellets often stirring them up. MD and HD fish in contrast fed well from the experiment outset. The FE value of the LD treatment should be regarded with some caution as the erratic feeding behaviour of the LD fish made accurate quantification of feed intake difficult. However; the low FE value for this group does provide an indication of the overall inefficient feeding behaviour of mulloway at low densities.

Qualitative observations during the present study did not identify any obvious agonistic behaviour among any of the density treatments while the similarity of growth heterogeneity between the density treatments reinforced this observation. This implies a moderate social hierarchy independent of the stocking densities used in this experiment (Brett, 1979). This also occurred despite the introduction of replacement fish to maintain density compliments.

One of the primary functions of shoaling behaviour in fish is predator avoidance (Pitcher, 1986) and the size of the shoal has been shown to directly influence the behaviour of individuals (Magurran & Pitcher, 1983). Magurran (1986) proposed that as a fish shoal increases in size, "corporate vigilance" for predators decreases. This relationship is not unique to fish and has been documented extensively in many animal behavioural studies (e.g. birds, Pulliam, 1973; wild boar, Quenette and Gerrard 1992; rabbits, Roberts, 1988; also see reviews by Lima & Dill, 1990; Roberts, 1996). The results and observations from this study indicate that a lower threshold of stocking density may also apply to mulloway; we hypothesize that, at a certain density, there forms a social cohesiveness which encourages a reduction in corporate vigilance and a change to normal feeding and behaviour. Below this threshold mulloway may become increasingly skittish and vigilant for (perceived) predators, increasing general activity and inefficient feeding behaviour. Growth and feeding studies combined with quantifiable behavioural data would test this hypothesis.

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FIGURE 1

Initial mean stocking weight (g) and mean weight of handled group over time (+/- se; n = 4). LD = 12, MD = 24 and HD = 48 fish aquaria⁻¹. Tukey-Kramer test on means between densities shown for each sampling period. Means sharing letters are not significantly different (P > 0.05).

Summary of initial and final data. Initial data are means \pm sd. Final data are pooled mean values (\pm se; n = 8) for each density tested. Tukey-Kramer test on means between densities shown as superscripts. Means sharing the same superscripts are not significantly different (P > 0.05).

Treatment	Survival (%)	Weight (g)	Length (mm)	Condition (K)	CV (%)	FE
Initial	-	16.5 (1.9)	117 (2.9)	1.04 (0.06)	11.2 (1.4)	-
Final LD	92.8 (3.3)	23.8 (0.7) ^a	131 (0.7) ^a	1.03 (0.02)	24.5 (2.3)	0.45 (0.04) ^a
MD	88.0 (2.5)	28.7 (0.8) ^b	139 (0.9) ^b	1.05 (0.01)	28.5 (1.9)	0.84 (0.03) ^b
HD	85.7 (1.9)	27.5 (0.7) ^b	138 (0.9) ^b	1.03 (0.01)	26.9 (1.2)	0.90 (0.04) ^b

Two-factor analysis of variance for survival, final weight, final length, condition (K), CV and FE. NS indicates not significant at P < 0.05, * significant at P < 0.05, ** significant at P < 0.01.

		Surviva (%)	al		Weig	ht _f		Lengtl	1 _f		Cond	lition	(K)	CV		F	ΥE		
Term	DF	MS	F	Р	MS	F	Р	MS	F	Р	MS	F	Р	MS	F	Р	MS	F	Р
A. Handled	v																		
Control	1	3.99	0.07	NS	1.39	0.31	NS	12.14	2.07	NS	0.00	0.05	NS	1.43	0.05	NS	0.01	0.60	NS
B. Density	2	105.06	1.79	NS	51.99	11.55	**	118.3	920.1	7 **	0.001	0.67	NS	30.13	1.08	NS	0.47	40.42	**
AB	2	61.65	1.05	NS	2.99	0.66	NS	1.79	0.30	NS	0.001	0.71	NS	43.39	1.55	NS	0.01	0.85	NS
Residual	18	58.56			4.50			5.87			0.001			27.99)				

4.5 Effect of stocking density and feeding time on the weight gain and performance of juvenile mulloway *Argyrosomus japonicus*

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1. INTRODUCTION

Mulloway *Argyrosomus japonicus*, belong to the family *Sciaenidae* and are commonly referred to as croakers and drums (Silberschneider & Gray, 2008). They are a relatively new but important aquaculture species in Australia. While there has been considerable research effort focused on other sciaenids such as red drum, aquaculture research on mulloway has only occurred in the last decade (Quartararo & O'Sullivan, 1994; Silberschneider & Gray, 2008). Recent studies in Australia have focused on the effects of abiotic factors such as feeding regime and photoperiod on growth of newly weaned fish (Fiedler & Bardsley, 1999; Ballagh, Pankhurst & Fielder, 2008), viability of aquaculture in saline ground water (Doroudi, Fielder, Allan & Webster, 2006) and metabolism (Fitzgibbon, Strawbridge & Seymour, 2007). However, little research has been published on either the nutritional requirements or best husbandry practices for this species.

From an economic point of view, two of the most critical and interlinked factors ensuring the success of an aquaculture farm are feeding practices and stocking density. Stocking density is known to affect the behaviour, growth and feed efficiency of fish reared in different culture systems (Kestemont & Baras, 2001; Tucker, Booth, Allan, Booth & Fielder, 2006). Preliminary studies have been published on appropriate feeding regimes or stocking densities for small mulloway grown in experimental aquaria (Ballagh et al., 2008; Pirozzi, Booth & Pankhurst, 2009), but no recommendations are available for mulloway grown in sea-cages. An early study on juvenile mulloway (0.5g) stocked into a sea-cage for 26 months reported harvest densities of approximately 10-15 kg m⁻³ (Quartararo, 1996), however the overall survival of fish in this trial was very poor ($\approx 26\%$). Pirozzi et al. (2008) demonstrated that juvenile mulloway stocked into small aquaria at finishing densities equivalent to 4.1 kg m⁻³ had significantly lower weight gain, poorer feed conversion ratio and exhibited a different feeding behaviour to fish held at either 8.2 or 16.3 kg m⁻³.

Fish species are generally classified as diurnal, nocturnal or crepuscular in their feeding habit (Bolliet, Azzaydi & Boujard, 2001; Chen, Umeda, Mitsuboshi & Hirazawa, 2007), however feeding fish correctly is often a difficult exercise, even when automated systems are used. This is because the feeding response is often variable and can be influenced by poorly understood endogenous mechanisms (Boujard, Gelineau & Corraze, 1995; Bolliet et al., 2001) or simply interrupted by physical conditions such as bad weather. In practice, farmed mulloway are known to be slow feeders and appear to prefer feeding on slow sinking pellets in low light conditions or in the evening rather than earlier in the day (Anthony O'Donohue, *pers. comm.*), but this observation has not be tested scientifically.

This objective of this study was to clarify the effects of stocking number and feeding regime on the weight gain and performance of juvenile mulloway reared in floating cages.

2. MATERIALS AND METHODS

2.1 Experimental plan

This experiment was designed to examine the interactive effects of stocking number and feeding regime (fixed factors) on the harvest weight and feeding performance of juvenile mulloway. Three stocking densities (20, 35 or 55 fish cage⁻¹) and three feeding regimes (1 feed at 0800h, one feed at 1600h or 2 feeds, one at 0800 and 1600h respectively) were established; hereafter AM, PM or AM-PM. Each treatment combination was randomly positioned within one of three 10 kL tanks (blocking factor) and replicate units were 200 L floating cages. Mean \pm sd of individual mulloway at the beginning of the experiment was 59 ± 2 g fish⁻¹ which equated to initial stocking densities of 5.9, 10.3 or 16.2 kg m⁻³.

2.2 Facilities

The experiment was carried out in a saltwater re-circulation system that consisted of 3 x 10 kL circular fibreglass tanks (3.4 m diameter x 1.2 m depth) housed within a plastic covered shade house at PSFI. Each of these tanks contained 9 cylindrical floating cages (dimensions approximately 0.2 m³; 0.6 m diameter x 0.7 m submerged depth) constructed of 10 mm perforated plastic mesh. Each cage was fitted with a lid to prevent the escape of fish (1.6 mm plastic mesh). Cages were firmly secured to the outer perimeter of the 10 kL tanks and remained in the same position during the entire experiment. Each 10 kL tank was provided with approximately 36-40 L min⁻¹ of pre-filtered (sand filter) water pumped from a combination bio-filter sump (5 kL). Effluent water from each tank drained through a 50 mm stand pipe and returned to the sump via gravity flow. Approximately 5% of the effluent water was discarded each day and replaced with clean disinfected estuarine water from a reservoir system. All 10 kL tanks were constantly aerated using a single 250 mm diameter circular air-pad diffuser. The floor of each 10 kL tank was vacuumed at least three times a week to ensure removal of accumulated faecal material and facilitate additional water exchange.

Water quality was monitored daily using a Model 611 electronic water quality analyser (Yeo-Kal Electronics, Brookvale, NSW, Australia). Total ammonia $[NH3 + NH4^+]$ was monitored using a rapid test kit procedure (Model 1.08024.0001, E. Merck, Darmstadt, Germany). During the experiment mean \pm sd of temperature, dissolved oxygen (DO₂), salinity or pH were $21 \pm 1^{\circ}$ C, $6.0 \pm 1 \text{ mg L}^{-1}$, $28 \pm 2 \%$ or $8.0 \pm 1 \text{ units}$. Total ammonia $[NH3 + NH4^+]$ was always $\leq 0.6 \text{ mg L}^{-1}$.

2.3 Fish

Mulloway were progeny of brood-stock held at the NSW DPI Fisheries Port Stephens Fisheries Institute (PSFI). Prior to use in the experiment the juveniles were reared in a large 10 kL tank and fed twice daily on a commercial marine finfish feed (Ridley Aqua-Feeds Pty. Ltd., Narangba, QLD: reported nutrient composition: 45% crude protein; 20% crude fat; 2.5% crude fibre). Prior to stocking the fish were given a prophylactic formalin bath (200 mg formaldehyde L^{-1}) then starved for 24 hours. Fish were then anaesthetised (20-30 mg L^{-1} ethyl- ρ -aminobenzoate), weighed in small groups and systematically distributed into 27 experiment cages. All fish in each of the 3 feeding regimes were carefully hand fed a commercially manufactured feed (Ridley Aquafeeds Pty Ltd) to apparent satiation 6 days per week (Monday – Saturday). Fish were not fed on Sundays. At the completion of the experiment (25 days) the fish in each cage were anaesthetised, weighed in small groups then returned to holding tanks. No fish died during the experiment.

2.4 Statistics

The effects of feeding regime and stocking density on the weight gain and feeding performance of juvenile mulloway was analysed using a non-additive factorial block design (Quinn & Keogh, 2002). The model consisted of two fixed factors (i.e. stocking number and feeding regime) and a random blocking factor to account for any unexplained variation in the response variable of interest due to differences between the three 10 kL tanks used to house the replicate cages (each of 9 treatments allocated to each tank). Data was assessed to ensure variances were homogeneous before conducting factorial ANOVA (Levene's test). Due to the short nature of the trial and the desire to increase the power of the experiment the significance level for all ANOVA and multiple comparisons tests (Tukey-Kramer test) was set $\alpha = 0.10$ and data was statistically analysed using NCSS (Hintze, 2006).

3. **RESULTS**

Table 1 describes the mean \pm pooled SEM for stocking weight and other selected performance criterion recorded at the end of the experiment. Juveniles gained between 16 to 20 g in body weight over the 25 day period and harvest densities reached an average of 7.46, 13.35 or 21.67 kg m⁻³, respectively. Relative feed intake varied between 1.16 to 1.52 % BW d⁻¹ and FCR ranged from 1.10 to 1.54:1.

There was no interaction effect of stocking density and feeding regime on harvest weight (P=0.9835), relative feed intake (P=0.7298) or FCR (P=0.5135) (Table 2). The harvest weight of juvenile mulloway was significantly affected by stocking number (P=0.0335) but not by feeding regime. Relative feed intake was strongly affected by feeding regime (P=0.0047) and by stocking number, but to a lesser extent (P=0.0521). FCR was significantly affected by stocking number (P=0.0314), but not feeding regime (P=0.1335) (Table 2).

Mulloway stocked at a density of 20 fish cage⁻¹ were significantly smaller after 25 days than fish stocked at densities of 35 or 55 fish cage⁻¹, respectively (Table 3). Feed intake was significantly higher in mulloway stocked at 20 fish cage⁻¹ compared to mulloway stocked at 35 fish cage⁻¹, but was not significantly different from mulloway stocked at a density of 55 fish cage⁻¹. Mulloway fed twice each day ate significantly more than fish fed once a day, irrespective of the time of feeding (Table 3). FCR was similar and significantly lower (better) in groups of mulloway stocked at 35 or 55 fish cage⁻¹ fish compared to mulloway stocked at 20 fish cage⁻¹.

4. DISCUSSION

This study has clearly demonstrated that stocking density has a strong influence on potential weight gain, relative feed intake and FCR in juvenile mulloway, at least over the short term. Importantly, this trial has also shown that weight gain is reduced and feed conversion ratio is increased (worse) when the selected stocking density is to low. In our case, this threshold density was 20 mulloway cage⁻¹ or a biomass of about 5.9 kg m⁻³. Relative feed intake was also strongly affected by feeding regime, with fish on the AM-PM treatment consuming significantly more than those on the other two treatments. Feeding to apparent satiation twice daily resulted in a 15% increase in relative feed intake above that recorded for fish fed only once a day. However this increase in feed consumption was not matched with an increase in weight gain, resulting in poorer FCR of fish reared under this regime. In addition, we found no difference between the relative feed intakes of mulloway fed in the morning or the afternoon and conclude that animals of this size exhibit no preferred feeding time, providing they are carefully hand fed.

The best FCR was recorded in mulloway stocked at 35 fish cage⁻¹ and fed at approximately 1600 h. These laboratory based values were also achieved without a significant reduction in harvest weight which

indicates that it may be more economical to stock juvenile mulloway at around 35 fish cage⁻¹ rather than at higher densities. In terms of biomass this intermediate range would reflect the start and harvest biomasses recorded in the present study of between 10 to 13.5 kgm⁻³. Our results suggest that densities as high as 22 kgm⁻³ are possible with no affect on weight gain, however there may be some trade-off in terms of poorer FCR.

These results support the work presented by Pirozzi et al. (2008) who also found the weight gain of juvenile mulloway was affected by stocking density. In their study 17g mulloway were stocked at densities of 12 (4.08 kgm⁻³), 24 (8.16 kg m⁻³) or 48 (16.32 kgm⁻³) fish per aquaria and on-grown for 36 days. At harvest they found that fish reared at the lowest density weighed less and had poorer FCR. The harvest weight and FCR of fish reared at the higher densities was much better and statistically similar. Interestingly, the harvest weight and FCR of fish reared at the intermediate density was numerically higher and lower, respectively. Like our results this suggests that an intermediate stocking density may be more appropriate for juvenile mulloway.

A survey of an existing mulloway farm in NSW found rearing densities in 100 m⁻³ net pens (5m x 5m x 5m) ranging from approximately 5 to 40 kgm⁻³. The number of fish per net pen ranged from about 1400 larger individuals (≈ 1.3 kg fish⁻¹) to 13,000 newly stocked animals (≈ 0.04 kg fish⁻¹). Rearing densities were based more on the movement of different generations or cohorts (size classes) through the farm system according to sales and available space as opposed to best husbandry practice. If the results of our intermediate stocking density are extrapolated to a farm situation, then the aforementioned 100 m⁻³ net pens could hold approximately 19,000 juvenile mulloway.

Juvenile mulloway weighing approximately 60g should be stocked into cages at starting densities > 6 kg m^{-3} to improve weight gain and FCR. Weight gain and FCR can be optimised at rearing densities closer to 10-13 kg m^{-3} . While higher stocking densities appear to be plausible there appears to be some reduction in FCR. This reduction may be indicative of the difficulty in effectively hand feeding a large number of fish to apparent satiation. This problem would tend to be exacerbated in an on-farm situation and thus some form of automated feeding system may be required. Small mulloway show no preference for feeding in the morning or the evening, at least under the conditions we described. This should give farmers of juvenile mulloway the confidence to feed their fish at either time of the day.

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	AM				PM		A			
Number fish cage ⁻¹	20	35	55	20	35	55	20	35	55	SEM
Stock weight g fish ⁻¹	56.40	57.60	59.80	58.30	59.00	59.70	57.80	59.80	59.60	0.90
Harvest weight g fish ⁻¹	73.30	76.00	76.90	74.70	78.50	79.70	74.80	78.50	79.80	2.00
Weight gain g fish ⁻¹	16.90	18.40	17.16	16.40	19.60	20.07	17.00	19.36	20.16	2.00
Stocking density kg m ⁻³	5.73	10.08	16.64	5.83	10.33	16.40	5.78	10.47	16.39	0.15
Harvest density kg m ⁻³	7.45	13.18	21.28	7.46	13.45	21.92	7.47	13.73	21.81	0.38
Total intake cage ⁻¹	420.50	674.00	1095.90	416.90	667.50	1129.90	498.10	790.10	1284.57	31.00
Intake % BW d ⁻¹	1.27	1.15	1.16	1.26	1.11	1.19	1.50	1.32	1.34	0.05
FCR	1.23	1.05	1.20	1.28	0.98	1.03	1.54	1.19	1.16	0.11
									_	

Mean \pm pooled SEM of mulloway fed nine different *feed regime x stocking density* treatments (n=3)

Results of factorial ANOVA on juvenile mulloway reared at three different stocking densities and fed one of three different feed regimes.

Performance criterion	df	SS	Mean square	F-ratio	P-value
Harvest weight (g fish ⁻¹)					
A: Stock number	2	105.51	52.75	8.93	0.0335*
B: Feed regime	2	33.66	16.83	1.34	0.3591
AB	4	2 965	0 741	0.09	0.9835
C: Block	2	48.67	24.33	0.02	0.9000
AC	4	23.63	5.907		
BC	4	50.33	12.58		
ABC	8	67.05	8.381		
hibe	0	07.05	0.501		
Feed intake (% BW d ⁻¹)					
A: Stock number	2	0.124	0.0624	6.76	0.0521*
B: Feed regime	2	0.228	0.1144	27.17	0.0047*
AB	4	0.009	0.0023	0.51	0.7298
C: Block	2	0.024	0.0121		
AC	4	0.037	0.0092		
BC	4	0.017	0.0042		
ABC	8	0.036	0.0045		
	-				
FCR					
A: Stock number	2	0.387	0.1933	9.28	0.0314*
B. Feed regime	$\frac{1}{2}$	0.185	0.0925	3 47	0 1335
AB	4	0.095	0.0237	0.89	0.5134
C [.] Block	2	0 200	0 1001	,	
AC	4	0.083	0.0208		
BC	4	0.106	0.0266		
ABC	8	0.213	0.0267		
nibe	U U	0.210	0.0207		

Results of multiple comparison tests for all pair-wise differences between the level means (n=9) of each factor.

		Performance indices		
Factor	-	Harvest weight (g fish ⁻¹)	Feed intake (% BWd ⁻¹)	FCR
Stock number	20	74.24 ^a	1.34 ^b	1.35 ^a
	35	77.92 ^b	1.19 ^a	1.07 ^b
	55	78.81 ^b	1.23 ^{ab}	1.13 ^b
	Std. error	0.81	0.03	0.05
Feed regime	AM	75.42	1.19 ^a	1.16
	PM	77.63	1.18^{a}	1.09
	AM-PM	77.92	1.38 ^b	1.29
	Std. error	1.18	0.02	0.05

4.6 Protein and energy utilization and the requirements for maintenance in juvenile mulloway (*Argyrosomus japonicus*)

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ABSTRACT

This study described the digestible protein (DP) and digestible energy (DE) utilization in juvenile mulloway and determined the requirements for maintenance. This was achieved by feeding triplicate groups of fish weighing 40 or 129 g held at two temperatures (20°C or 26°C) a commercial diet (21.4 g DP MJ DE⁻¹) at four different ration levels ranging from 0.25% initial body weight to apparent satiation over 8 weeks. Weight gain and protein and energy retention increased linearly with increasing feed intake. However, energy retention efficiency (ERE) and protein retention efficiency (PRE) responses were curvilinear with optimal values, depending on fish size, approaching or occurring at satiated feeding levels. Maximum predicted PRE was affected by body size but not temperature; PRE values were 0.50 and 0.50 for small mulloway and 0.41 and 0.43 for large mulloway at 20°C and 26°C respectively. ERE demonstrated a similar response; with values of 0.42 and 0.43 for small and 0.32 and 0.34 for large mulloway at 20°C and 26°C respectively. Utilization efficiencies for growth based on linear regression for DP (0.58) and DE (0.60) were independent of fish size and temperature. The partial utilization efficiencies of DE for protein (k_p) and lipid (k_l) deposition estimated using a factorial multiple regression approach were 0.49 and 0.75 respectively. Maintenance requirements estimated using linear regression were independent of temperature for DP (0.47g DP kg^{-0.7} day⁻¹) while maintenance requirements for DE increased with increasing temperature (44.2 to 49.6 kJ DE kg^{-0.8} day⁻¹). Relative feed intake was greatest for small mulloway fed to satiation at 26°C and this corresponded to a greater increase in growth. Large mulloway fed to satiation ate significantly more at 26°C but did not perform better than the corresponding satiated group held at 20°C. Mulloway should be fed to satiation to maximize growth potential if diets contain 21.4 g DP MJ DE⁻¹.

1. INTRODUCTION

The utilization of digestible protein (DP) and digestible energy (DE) by growing animals is dependant on the composition of the diet and the efficiency with which deposition occurs (van Milgen and Noblet, 2003; Schroeder and Titgemeyer, 2008). In fish, patterns of protein deposition with increasing levels of DP intake vary considerably between species, diet and experimental conditions and responses have been described as linear (Lupatsch et al., 2001; Fournier et al., 2002; Lupatsch and Kissil, 2005; Peres and Oliva-Teles, 2005) or curvilinear (Huisman et al., 1979; McGoogan and Gatlin, 1998; Watanabe et al., 2000b; Bureau et al., 2006). These responses indicate that the utilization efficiencies are either constant or tend to plateau with increasing protein intake. Unfortunately, such variations emphasize the need to determine nutrient retention profiles and utilization efficiencies of growing fish on a species by species basis. Understanding how nutrients are utilized is an essential step towards developing bioenergetic models that predict growth responses, feeding requirements and nutrient losses to the environment (Bureau et al., 2002).

The concept of maintenance requirements is one that may be considered as paradoxical with regard to growing animals but it is a concept that has proved useful for animal nutritionists because it allows the partitioning of production and maintenance costs based on the assumption that the two are additive (van

Milgen et al., 2000; Bureau et al., 2002). Maintenance DE requirements for fish have been shown to range from 32 - 77 kJ DE kg^{-0.8} day⁻¹ (Watanabe et al., 2000a; Lupatsch and Kissil, 2005) and vary depending on temperature, species and fish size. Published maintenance requirement values for DP are less common in the literature but values of 0.45 - 0.96 g DP kg^{-0.7} day⁻¹ have been recorded (Lupatsch et al., 1998; Lupatsch and Kissil, 2003; Peres and Oliva-Teles, 2005; Glencross, 2008).

The objectives of this study were to determine i) the protein and energy utilization responses to increasing DE and DP intake, ii) the efficiencies of DP and DE utilization, and iii) the maintenance requirements of juvenile mulloway. This was achieved using two size classes of mulloway (40 or 129 g) at two temperatures (20° C or 26° C).

2. MATERIALS AND METHODS

The protein and energy utilization of mulloway was tested by feeding four different ration levels ranging from 0.25% of initial body weight (ibw) using a commercial diet (Ridley AquaFeed Pty. Ltd., Narangba, Qld. Australia) to two size treatments (small or large; ibw (mean \pm SD) = 40.2 \pm 5.7 g and 129.3 \pm 17.2 g) at two temperatures (20 or 26°C). The experiment was run over 8 weeks using fish produced at the New South Wales Department of Primary Industries, Port Stephens Fisheries Institute (PSFI). Fish were stocked into 200 l white opaque tapered cylindrical tanks (Dimensions: Top diameter = 78 cm; Bottom diameter = 68 cm; Height = 55 cm) at 40 small or 12 large fish tank⁻¹. Mulloway are a gregarious species and stocking densities were chosen to optimize growth potential (Pirozzi et al., 2009). Each size and ration treatment were randomly assigned to triplicate tanks within each temperature treatment with each tank constituting an experimental unit.

2.1 Experiment design

The protein and energy utilization of mulloway was tested by feeding four different ration levels ranging from 0.25% of initial body weight (ibw) using a commercial diet (Ridley AquaFeed Pty. Ltd., Narangba, Qld. Australia) to two size treatments (small or large; ibw (mean \pm SD) = 40.2 \pm 5.7 g and 129.3 \pm 17.2 g) at two temperatures (20 or 26°C). The experiment was run over 8 weeks using fish produced at the New South Wales Department of Primary Industries, Port Stephens Fisheries Institute (PSFI). Fish were stocked into 200 l white opaque tapered cylindrical tanks (Dimensions: Top diameter = 78 cm; Bottom diameter = 68 cm; Height = 55 cm) at 40 small or 12 large fish tank⁻¹. Mulloway are a gregarious species and stocking densities were chosen to optimize growth potential (Pirozzi et al., 2009). Each size and ration treatment were randomly assigned to triplicate tanks within each temperature treatment with each tank constituting an experimental unit.

2.2 Experiment system

The experiment system consisted of two separate 1700L recirculating bio-filtration units each supplying 24 x 200 L replicate tanks (each unit total volume = 6500 L). The temperature for each unit was controlled with a chiller and heater in an antagonistic mode which allowed precise temperature control of $\pm 0.5^{\circ}$ C of the set temperature. All fish were initially stocked at 23°C and the temperature adjusted 1°C day⁻¹ until the experiment temperatures were reached. Flow to each tank was approximately 4 L min⁻¹ and orientated in a way to create a weak centripetal current which allowed the retention of feed pellets in the tank while removing faeces via a central upright 32mm diameter pvc overflow pipe which was fixed approximately 1cm off the bottom of each tank. Black plastic sheets were placed around each tank and across the top front half to minimise disturbance. All tanks were exposed to indirect natural light (photoperiod 13L:11D). Ammonium (NH₄⁺) (<0.1 mg/L), dissolved oxygen (>5.0 mg/L), pH (7.5 - 7.8) and salinity (30 - 34 ppt) were monitored regularly throughout the duration of the experiment.
2.3 Feed and feeding

Proximate composition of the diet was $(g kg^{-1})$: 961 dry matter; 90 ash; 455 crude protein; 187 fat and 22.2 MJ kg⁻¹ gross energy. The apparent digestibility co-efficient for protein was 0.88 and energy was 0.84 (Booth, unpublished data 2008).

Fish were fed 6 mm extruded sinking pellets from once up to four times daily depending on ration size to improve the likelihood of all fish obtaining pellets in the lower ration treatments or to maximise voluntary daily feed intake in the higher ration treatments. Any uneaten pellets were counted then siphoned from tanks approximately 45 min after initial feeding. Total daily feed intake was adjusted accordingly (predetermined individual pellet weight mean \pm SD = 0.21 \pm 0.02 g; n = 202). The commercial feed used in this study had excellent water stability and it was assumed nutrient losses through leaching were insignificant.

2.4 Sample preparation and analyses

Fish were fasted for 48 h prior to sampling for carcass composition. Initial representative samples of 10 fish of each size class were collected before the start of the experiment and frozen (-20°C). At the conclusion of the feeding trial all fish were euthanized with an overdose of benzocaine (ethyl-p-aminobenzoate), weighed and stored frozen for compositional analyses. Compositional changes in energy, lipid, ash and moisture were estimated by comparing the initial fish carcass samples with those from the feeding trial. Estimates of initial whole body protein were based on the compositional value of 191.4 g kg⁻¹. This value was derived in a separate study by Pirozzi, Booth and Allan (unpublished data, 2008) to establish the compositional profile of mulloway where several hundred fish were sampled in groups representing size classes ranging from 2 - 2100 g (n = 3 - 100 fish per group depending on size). Using this value was necessary because of a data transcription error with the original initial values for protein composition. All other initial compositional constituents appeared to be true representations of the initial carcass composition. Assuming a fixed initial whole body protein composition is valid as the proportional relationship between body protein and body weight in fish is known to be relatively constant (Shearer, 1994; Lupatsch et al., 1998; Dumas et al., 2007).

Whole carcass composition was determined by placing the weighed fish into 5 l glass beakers, covering with aluminum foil and then autoclaving for 99 min at 121°C. After cooling to room temperature any changes in weight were accounted for and assumed to be changes in moisture content. The samples were then homogenised in situ with a hand blender and a sub-sample taken for dry matter determination. A portion of the remaining homogenate was then transferred to plates and oven dried at approximately 80°C. The desiccated samples were then finely ground in a laboratory blender and analysed in accordance with AOAC (2005). Protein was calculated from total nitrogen based on N x 6.25 using the Dumas method. Dry matter was calculated gravimetrically after oven drying at 105°C. Ash was calculated gravimetrically. Gross energy was determined by adiabatic bomb calorimetry. Fat was measured gravimetrically after chloroform-methanol extraction.

The following performance indices were calculated for each treatment group:

Daily weight gain (g fish⁻¹ day⁻¹) = Final body weight (fbw) -ibw / number of days

Daily protein gain (g fish⁻¹ day⁻¹) = Final carcass protein content – 0.1914 x initial sample bw / number of days

Daily energy gain (kJ fish⁻¹ day⁻¹) = Final carcass energy content – initial carcass energy content / number of days

Feeding Efficiency (FE) = Weight gain / Total feed intake

Protein Retention Efficiency (PRE) = Protein gain / Total DP intake

Energy Retention Efficiency (ERE) = Energy gain / Total DE intake

Data are also expressed as geometric mean body weights (GMBW) and scaled using the metabolic body weight exponent value of 0.7 for protein retention data and 0.8 for energy retention data (after Brett and Groves, 1979; Lupatsch et al., 1998).

2.5 Data analyses

The effects of varying feed ration (fixed; 4 levels) at different temperatures (fixed; 2 levels) on performance indices and compositional data were tested with 2-way ANOVA for each size class (fbw was not a significant co-variant for analyses of compositional data). Formal comparisons using ANOVA were not made between sizes as ration levels were not orthogonal. Normality of the data was checked with skewness, kurtosis and omnibus normality tests. Assumptions of homogeneity of variances were tested using modified Levenes' equal variance test. Tukey-Kramer test was used for *a posteriori* multiple comparison of means on significant terms. All results were regarded as significant at p<0.05. Data were normally distributed for all performance indices for large fish at both temperatures. Data were non-normally distributed for FE, PRE and ERE for small fish at 20°C and for FE and PRE for small fish at 26°C; these data could not be normalized. ANOVA was still performed and due regard should be given to subsequent interpretations of the results. All performance indices and carcass composition data variances were homogenous (Levene's; p>0.05).

Nonlinear regression was applied to PRE data where the asymptote of the quadratic function was considered as the optimal daily dietary DP intake giving the maximum predicted PRE value (Shearer, 2000).

Daily maintenance requirements for dietary protein and energy at 20 and 26° C were estimated using linear regression of daily intake and gain where the *x*-intercept describes the daily requirement for maintenance, the slope of regression describes the utilization efficiency and the reciprocal of the slope describes the nutrient cost of production.

Partial energy efficiencies for protein and lipid deposition were further investigated using the factorial method based on Kielanowski (1965) where DE intake can be partitioned as:

DE intake (kJ kg^{-b} day⁻¹) = DE_m + PD/ k_p + LD/ k_l

Where $DE_m = daily$ maintenance energy requirement (kJ DE kg^{-b} day⁻¹); PD = energy retained as protein (kJ day⁻¹); $k_p = partial$ energy efficiency for protein deposition; LD = energy retained as lipid (kJ day⁻¹); k_1 = partial energy efficiency for lipid deposition. The metabolic weight exponent (b) was estimated simultaneously with the above parameters which gave values of 0.817(±0.05) and 0.784(±0.07) at 20 and 26°C respectively. These values were statistically indistinguishable from the common inter-specific exponent value of 0.8 applied to energy metabolism of teleost fishes (Clarke & Johnston, 1999). A fixed exponent value of 0.8 was therefore used in the model to estimate parameters DE_m, k_p and k_1 . Least squares

regression method assumes normally distributed residuals therefore robust multiple regression was used which minimizes the influence of outliers on coefficient estimates (Montgomery and Peck, 1992).

The heat of combustion values for protein and lipid required to determine PD and LD (above) were also derived using robust multiple regression however with the *y*-intercept term removed from the regression model:

 $\operatorname{RE}(\mathrm{kJ}) = a \ge \mathrm{RP} + c \ge \mathrm{LD}$

Where RE = retained energy (kJ); a = protein heat of combustion (kJ g^{-1}); c = lipid heat of combustion (kJ g^{-1}); PD = protein deposition (g); LD = lipid deposition (g)

3. **RESULTS**

Survival at the end of the experiment was 100%. The effect of ration on weight, protein and energy gain in small mulloway varied significantly depending on temperature (Table 1). Weight, protein and energy gain were significantly higher at the highest ration level at 26°C for small mulloway (Table 2). Ration level but not temperature affected the weight, protein and energy gain of large mulloway (Table 1). Large mulloway fed to satiation at 26°C also demonstrated higher gain but this was significantly different from the satiated treatment at 20°C only for protein retention (Table 2). Relative feed intake (RFI) was significantly greater at the satiated level for small mulloway at 26°C (p<0.05) with 6.1±0.2 g kg^{-0.7} day⁻¹ at 20°C. RFI was also significantly greater for large mulloway at 26°C (p<0.05) with 5.4±0.1 g kg^{-0.7} day⁻¹ consumed compared to 4.4±0.2 g kg^{-0.7} day⁻¹ at 20°C.

3.1 Utilization efficiencies and maintenance requirements

The effect of ration on FE varied significantly depending on temperature for small mulloway while both ration and temperature significantly, but independently, affected FE for large mulloway (Table 1). PRE was significantly influenced by ration but not temperature in small mulloway while the influence of ration on PRE in large mulloway was dependent on temperature (Table 1). The interaction occurred because of the relatively better PRE value at 26°C compared to 20°C when large mulloway were fed the lowest ration level (Table 2). ERE in small and large mulloway was significantly influenced by ration but not temperature (Table 1).

PRE in mulloway demonstrated a curvilinear response to increasing DP intake. Temperature had little effect on the maximum predicted PRE provided that DP intake was increased with increasing temperature (Figure 1). Body size, however, did influence PRE (Table 2) with the maximum predicted PRE for small mulloway 0.50 and 0.50 and large mulloway 0.41 and 0.43 at 20°C and 26°C respectively. The daily protein intake to achieve maximum predicted PRE was 1.7 and 2.0 g DP kg^{-0.7} day⁻¹ at 20°C and 26°C respectively (Figure 1). The relationship between digestible protein intake (g DP kg^{-0.7} day⁻¹) and PRE can be described as:

$PRE (20^{\circ}C) = -0.542 + 1.162DP - 0.337DP^{2}$	$(r^2 = 0.89, n = 24)$	1.
$PRE (26^{\circ}C) = -0.418 + 0.887DP + -0.222DP^{2}$	$(r^2 = 0.87, n = 24)$	2.

Energy retention efficiency demonstrated a similar curvilinear response (Figure 2) with increasing DE intake and body weight greatly influencing the efficiency values (Table 2). Temperature did not affect the maximum predicted ERE values with 0.42, 0.44 and 0.32, 0.34 for small and large mulloway at 20°C and

 26° C respectively. The relationship between digestible energy intake (kJ DE kg^{-0.8} day⁻¹) and ERE can be described as:

ERE $(20^{\circ}C) = -1.121 + 0.025DE - 0.0001DE^2$ $(r^2 = 0.94, n = 24)$ 3. ERE $(26^{\circ}C) = -1.128 + 0.028DE - 0.0001DE^2$ $(r^2 = 0.97, n = 24)$ 4.

The daily energy intake (kJ DE kg^{-0.8} day⁻¹) to achieve a maximum predicted ERE was 107 kJ DE kg^{-0.8} day⁻¹ at 20°C and 125 kJ DE kg^{-0.8} day⁻¹ at 26°C (Figure 2).

There was no temperature (p>0.5) or size effect (p>0.1) between the slopes of regression when considering the relationship between protein intake (g DP kg^{-0.7} day⁻¹) and protein gain (g kg^{-0.7} day⁻¹) (Figure 3). A pooled value of 0.58±0.02 describes the utilization efficiency of protein for mulloway at 20 - 26°C. The relationship between protein gain (g kg^{-0.7} day⁻¹) and DP intake (g kg^{-0.7} day⁻¹) was linear and can be described as:

Protein gain =
$$0.581$$
DP - 0.272 ($r^2 = 0.97, n = 48$) 5.

The corresponding cost of DP per unit of protein gain was 1.72 g g⁻¹. Temperature did not have a significant effect on maintenance requirements for protein with the greatest difference of <0.1 g fish⁻¹ (Figure 3). Estimates of maintenance protein requirements for mulloway held at 20 - 26°C are 0.47 g DP kg^{-0.7} day⁻¹.

There was no temperature (p>0.1) or size effect (p>0.5) between the slopes of regression when considering the relationship between energy intake (kJ DE kg^{-0.8} day⁻¹) and energy gain (kJ DE kg^{-0.8} day⁻¹) (Figure 4). A pooled value of 0.60±0.01 describes the utilization efficiency of energy for mulloway at 20 - 26°C. The corresponding cost of DE per unit of energy gain is 1.66 kJ kJ⁻¹. The relationship between energy intake (kJ kg^{-0.8} day⁻¹) and energy gain (kJ kg^{-0.8} day⁻¹) was linear and can be described as:

Energy gain $(20^{\circ}C) = 0.604DE - 26.689$	$(r^2 = 0.98, n = 24)$	6
Energy gain $(26^{\circ}C) = 0.591DE - 29.302$	$(r^2 = 0.98, n = 24)$	7

The maintenance requirements for energy (kJ DE kg^{-0.8} day⁻¹) varied significantly depending on temperature (p < 0.005) (Figure 4). Estimates of maintenance energy requirements were 44.2 and 49.6 kJ DE kg^{-0.8} day⁻¹ at 20°C and 26°C respectively.

Heat of combustion values derived using robust multiple regression analysis were 22.9 and 37.0 kJ g⁻¹ for protein and lipid respectively. Partial energy efficiencies estimated using the factorial method were $k_p = 0.49 \pm 0.09$ and $k_1 = 0.75 \pm 0.19$. Therefore, based on k_p , k_1 and the heat of combustion values for protein and lipid the energetic cost to mulloway to deposit 1 g of protein is 46.73 kJ and 1 g of lipid is 50.0 kJ.

 DE_m was estimated at 43.0 ± 3.8 kJ kg^{-0.8} day⁻¹ using the factorial method and compared well with estimates of 46.60 ± 1.21 kJ DE kg^{-0.8} at zero energy gain (*x*-intercept) derived using linear regression of combined temperature and size data (Figure 4).

Figure 5 shows the response of energy gain as a function of DE intake partitioned between protein energy (PE) and lipid energy (LE) where LE is calculated as the difference between total energy gain and PE gain and assumes no other contributing non-protein energy. There was no significant difference found between the slopes (p>0.1) when comparing PE and LE. There was no significant difference found when comparing the slopes (p>0.1) and y-intercepts (p>0.1) between PE deposition at 20°C or 26°C. A common

linear regression can describe the relationship between PE deposition (kJ kg^{-0.8} day⁻¹) and DE intake (kJ kg^{-0.8} day⁻¹) at these temperatures:

PE gain =
$$0.288\text{DE} - 8.213$$
 ($r^2 = 0.98, n = 48$) 8.

There was no temperature effect between slopes (p>0.5) with regard to LE deposition however the *y*-intercepts differed significantly (p<0.0001). The relationship between LE deposition (kJ kg^{-0.8} day⁻¹) and DE intake (kJ kg^{-0.8} day⁻¹) can be described as:

LE gain (20°C) = 0.304DE -17.960 $(r^2 = 0.97, n = 24)$ 9. LE gain (26°C) = 0.310DE - 21.280 $(r^2 = 0.98, n = 24)$ 10.

3.2 Effect of ration level and temperature on carcass composition

Protein content was significantly lower in the 20°C treatment compared to the 26°C treatment for both size mulloway while large mulloway protein content was also significantly affected by ration level although this occurred independent of temperature (Table 1). The average overall difference between temperature treatments within sizes for protein content, although statistically significant, was <10 g kg⁻¹.

Energy content was significantly affected by ration level but not temperature (Table 1) and there was a trend for energy content to increase with increasing ration (Table 2).

Lipid content generally increased with increasing ration level. The effect of feed ration on lipid composition varied significantly with temperature (Table 1) and the interaction occurred because lipid content at the lowest rations (Ration level 1 for small fish; Ration level 1 and 2 for large fish) was, on a relative basis, less in the 26°C treatment compared to the 20°C treatment while at the higher ration levels the opposite occurred; lipid content was greater in the 26°C treatment (Table 2).

Ash content in small mulloway demonstrated a similar, but opposite, response to lipid content (Table 2). Ash content in large mulloway tended to be higher at 26°C and decrease with increasing ration level (Table 2).

Both temperature and ration level significantly, but independently, affected moisture content in small and large mulloway (Table 1).

4. **DISCUSSION**

Understanding how growth is affected by the ration level of a particular diet is important in optimizing feeding strategies for aquaculture species. A curvilinear response dictates that feeding restricted rations will optimise growth and maximise feeding efficiencies while reducing waste outputs and increasing overall cost effectiveness. Conversely a linear response determines feeding to satiation to achieve maximal growth and feeding efficiencies. Growth and protein deposition in mulloway demonstrated a linear response (Figure 3) while optimal retention efficiencies, depending on size, approached or were at satiated intake levels (Figure 1). The largest difference in PRE between the predicted optimal and satiated DP intake level occurred with small mulloway at 26°C with a difference of only 0.07 g DP fish⁻¹ day⁻¹. The commercial diet used in this study, which is also commonly used by farmers in Australia, should therefore be fed to satiation to maximize growth potential and feeding efficiencies in mulloway.

At 0.60, the energy utilization efficiency of mulloway is within the range reported for other fish species (0.4 - 0.7; see Bureau et al., 2006). No significant differences were found between the energy utilization

efficiencies of the two size classes of mulloway or temperatures used in this study. These observations have been similarly demonstrated for European seabass (*Dicentrarchus labrax*) (Lupatsch et al., 2001), Asian seabass (also known as barramundi, *Lates calcarifer*) (Lupatsch and Kissil, 2003) and rainbow trout (*Oncorhynchus mykiss*) (Azevedo et al., 1998). However, it may be that the ranges between the sizes and temperatures studied were not sufficient to observe a shift in utilization efficiencies. Glencross (2008) demonstrated an improvement in the energy utilization efficiencies for growth with increasing size of barramundi of 0.61 for 15 g fish to 0.76 for 410 g fish although the regression model appeared heavily influenced by the data set from the satiated group of small fish in that study. This marked difference in the utilization efficiency of dietary energy with size has important implications in bioenergetic modelling and feed formulations for the species and warrants further investigation.

The protein utilization efficiency of mulloway was 0.58 and independent of temperature and size. Similar values and temperature effects have been demonstrated with European seabass (0.52; 20-26°C) (Lupatsch et al., 2001), barramundi (0.49-0.51; 21-30°C) (Lupatsch and Kissil, 2003; Glencross, 2008) and white grouper (*Epinephelus aeneus*) (0.54; 22-27°C) (Lupatsch and Kissil, 2005). Peres and Oliva-Teles (2005) demonstrated a protein utilization efficiency of 0.64 for European seabass which is higher than that reported by Lupatsch et al. (2001). Differences in protein utilization efficiencies can, in part, be accounted for by the amino acid composition of the diet (Sandberg et al., 2005).

The partial energy retention efficiency of PE (protein energy) in mulloway ($k_p = 0.49$) was similar to and falls within the SEM ranges of k_p values recorded for other carnivorous fish species such as gilthead seabream (*Sparus aurata*) (0.53), European seabass (0.53), white grouper (0.56) (Lupatsch et al., 2003) and rainbow trout (0.53) (Azevedo et al., 2005).

 k_1 values for mulloway (0.75) are directly comparable to gilthead seabream (0.76) (Lupatsch et al., 2003) but are lower than those recorded for European seabass (0.91) and white grouper (0.91) (Lupatsch et al., 2003) although k_1 for mulloway is still within the lower SEM range of these two species. Lupatsch et al. (2003) suggested that PE was also used in lipid deposition at higher PE intake levels in gilthead seabream hence the lower k_1 value for that species and supported this argument by demonstrating the non-linear response of PE deposition with increasing PE intake. However, the relationship between PE deposition and PE intake in mulloway was linear ($r^2 = 0.98$). k_1 values of approximately 0.9 can be expected if dietary lipid is the base nutrient for body lipid synthesis (Emmans, 1994; van Milgen et al., 2001; Lupatsch et al., 2003) however, if lipids are also synthesised from dietary energy supplied by carbohydrates then a reduction in k_1 may be seen. The commercial diet used in this study contained 268 g kg⁻¹ nitrogen–free extract (NFE = 100 – (protein + lipid + ash) suggesting that non-lipid dietary energy was available for lipid synthesis and may, in part, explain the relatively lower k_1 value.

The proportional rate of deposition of protein:lipid remained relatively constant with increasing DE intake for mulloway with LE deposited at a slightly numerically greater rate (p>0.1) than PE (Figure 5). This is in contrast to rainbow trout which show a clear decrease in protein:lipid deposition with increasing DE intake (Rodehutscord and Pfeffer, 1999; Bureau et al., 2006). The difference between the partial energy utilization efficiencies of protein and lipid in mulloway may not be of a sufficient magnitude to demonstrate a clear protein sparing effect if dietary lipid levels were to be increased. This has been demonstrated by Pirozzi et al. (2010) where mulloway fed increasing levels of DP at either one of two fixed DE levels (16 or 21 MJ kg⁻¹) showed no obvious protein sparing effect.

Values for maintenance energy requirements ranged from 44.2 to 49.6 kJ DE kg^{-0.8} day⁻¹ depending on temperature and fall within the maintenance DE values common to other fish species (40 – 60 kJ DE kg^{-0.8} day⁻¹; Bureau et al., 2002). Protein requirements for maintenance for mulloway were 0.47 g DP kg^{-0.7} and were found to be independent of temperature. Similar protein maintenance requirements ~0.45 g DP kg^{-0.7}

day⁻¹ (Lupatsch and Kissil, 2003; Glencross, 2008) and temperature effects (Lupatsch and Kissil, 2003) have also been demonstrated in barramundi.

Feeding at maintenance energy level does not necessarily imply that a constant body weight is maintained. Pigs have been shown to maintain zero energy retention while depositing protein and gaining body weight at the expense of body lipid (Ledividich et al., 1980). In the current study the *y*-intercept for PE (equation 8) was much larger than for LE (equations 9 & 10) therefore a DE intake at or slightly above maintenance level will yield positive protein deposition in mulloway without lipid gain. This has been demonstrated in yellowtail (*Seriola quinqueradiata*) (Watanabe et al., 2000b), European seabass (Peres and Oliva-Teles, 2005) and rainbow trout (Bureau et al., 2006) fed at or near maintenance rations. These observations support the principle that weight gain in growing animals is driven by protein deposition (van Milgen et al., 2000; Bureau et al., 2002). The separation between LE deposition (Figure 5) accounts for the different requirements for total energy at different temperatures (Figure 4) and indicates that lipids rather than protein are mobilised as an energy source to meet the increased maintenance energy demands imposed at higher temperatures. This allows the growing animal to continue to deposit protein at a rate predetermined by its genetic potential.

The improved growth rates in small mulloway can be directly attributed to a proportional increase of feed intake at the satiated level. However, large mulloway fed to satiation ate significantly more at 26°C than 20°C but did not demonstrate significantly better growth. This discrepancy may be attributed to the relatively greater costs for maintenance imposed on large mulloway at the higher temperature and may also indicate a shift in the DP:DE requirements for larger fish. Brett (1971) and Kellogg and Gift (1983) suggest that the final temperature preference exhibited by fish coincides with the temperature required to optimize biochemical and physiological processes. In a temperature for 20g mulloway. The improved protein and energy retention of mulloway fed to satiation at 26°C in the current study tends to support those findings, particularly for small mulloway. However, at satiated levels the maximum retention efficiencies of dietary energy to be greater at 20°C (Figure 2) which is likely to be related to the reduced maintenance energy requirements at that temperature. This suggests that improved growth rates can occur at 26°C provided that dietary intake is optimized.

The effect of ration on whole-body composition was however independent of temperature except for lipid (small and large mulloway) and ash (small mulloway) (Table 1). Lipid content is known to vary directly depending on intake levels (Shearer, 1994) and as lipid levels changed in mulloway a corresponding change in ash and moisture was also observed. This can be attributed to a proportional shift between bone and muscle mass with increasing feed intake. Whole-body protein content is considered to remain constant and independent of feed intake and temperature (Shearer, 1994); however, protein content in mulloway was shown to vary significantly with temperature (small and large fish) and ration (large fish). The overall difference in protein content between significant treatment levels was, however, quite small at <10 g kg⁻¹ on average. It can therefore be concluded that while feed intake and temperature have a statistically observable effect on the protein composition of mulloway, protein levels do indeed remain fairly well conserved. This result also supports the use of a constant value for the initial fish protein composition (191.4 g kg⁻¹) which, when compared across all combined size, temperature and ration treatment values, differed by <10 g kg⁻¹.

To summarize, the utilization efficiencies of DE (0.60) and DP (0.58) for growth in mulloway were shown to be constant and independent of fish size, ration level or temperature used in this study. The partial utilization efficiencies of DE for protein (k_p) and lipid (k_l) deposition were 0.49 and 0.75 respectively. Maintenance requirements for protein (0.47 g DP kg^{-0.7} day⁻¹) were influenced by body size but were

independent of temperature while maintenance requirements for energy increased with increasing temperature (44.21 to 49.59 kJ DE kg^{-0.8} day⁻¹) and were also influenced by body size. Mulloway should be fed to satiation to maximize growth potential if diets contain 21.4 g DP MJ DE⁻¹.

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Two-way ANOVA on performance indices and carcass composition (as received basis) for both small and large mulloway. ns = not significant at p<0.05, * = p<0.05, ** = p<0.01

	Source of Variation											
Variable		Smal	1	Large								
	Temp.	Ration	Interaction	Temp.	Ration	Interaction						
Performance Indices												
Weight gain	ns	**	**	ns	**	ns						
Protein gain	ns	**	**	ns	**	ns						
Energy gain	ns	**	**	ns	**	ns						
FE	**	**	**	*	**	ns						
PRE	ns	**	ns	ns	**	**						
ERE	ns	**	ns	ns	**	ns						
Carcass Composition												
Protein	**	ns	ns	**	**	ns						
Lipid	ns	**	**	ns	**	**						
Moisture	**	**	ns	**	**	ns						
Ash	**	**	**	**	**	ns						
Energy	ns	**	ns	ns	**	ns						

Summary of performance indices and carcass composition of small and large mulloway held at 20°C or 26°C. Tukey-Kramer test on means between temperature treatments within each size class. Means sharing superscripts are not significantly different (p>0.05).

	20°C Small					26°C	Small		20°C Large				26°C Large			
		Feed	Ration		Feed Ration				Feed Ration				Feed Ration			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Performance Indicies																
Initial Body Weight (g)	40.2	40.0	40.3	39.6	39.9	40.3	40.4	40.8	124.6	126.1	127.5	134.1	132.1	131.7	131.0	127.5
Final Body Weight (g)	39.2 ^a	59.1 ^b	73.3 ^c	74.7 ^c	35.7 ^a	54.4 ^b	70.5 ^c	83.7 ^d	121.8 ^a	150.7 ^c	163.6 ^{cd}	180.3 ^{de}	127.9 ^{ab}	147.4 ^{bc}	164.5 ^{cde}	184.3 ^e
Gain (g/fish/day)	-0.02 ^a	0.33 ^c	0.57 ^d	0.61 ^d	-0.07 ^a	0.24 ^b	0.52 ^d	0.74 ^e	-0.05 ^a	0.43 ^{bc}	0.63 ^{bd}	0.81 ^{de}	-0.07 ^a	0.27 ^b	0.59 ^{bcd}	1.00 ^e
Feed Intake (g/fish/day)	0.09	0.37	0.59	0.66	0.09	0.37	0.60	0.84	0.29	0.72	1.05	1.20	0.31	0.75	1.08	1.44
FE	-0.20 ^b	0.90 ^c	0.96 ^c	0.92 ^c	-0.79 ^a	0.66 ^c	0.87 ^c	0.88 ^c	-0.17 ^a	0.60^{bc}	0.60 ^{bc}	0.66 ^c	-0.24 ^a	0.36 ^b	0.54 ^{bc}	0.69 ^c
DP Intake (g/fish/day)	0.04	0.15	0.24	0.26	0.04	0.15	0.24	0.33	0.12	0.29	0.42	0.48	0.12	0.30	0.43	0.57
Protein Retention (g/fish/day)	-0.01 ^a	0.06^{b}	0.11 ^c	0.12 ^c	-0.01 ^a	0.06^{b}	0.11 ^c	0.15 ^d	-0.01 ^a	0.11 ^{bc}	0.15 ^{bcd}	0.20 ^d	0.01 ^a	0.10^{b}	0.17 ^{cd}	0.25 ^e
PRE	-0.20^{a}	0.43 ^b	0.48^{b}	0.46^{b}	-0.26^{a}	0.38 ^b	0.45^{b}	0.46^{b}	-0.05^{a}	0.38 ^c	0.36 ^c	0.41 ^c	0.12 ^b	0.32 ^c	0.40°	0.43 ^c
DE Intake (kJ/fish/day)	1.71	6.80	11.08	12.28	1.69	6.84	11.09	15.55	5.37	13.36	19.54	22.35	5.70	14.01	20.09	26.81
Energy Retention (kJ/fish/day)	-1.09 ^a	2.02^{b}	4.35 ^c	4.53 ^c	-1.39 ^a	1.67^{b}	3.98 ^c	6.04 ^d	-3.20^{a}	1.58 ^{bc}	4.05 ^c	6.13 ^{cd}	-3.05^{a}	0.37^{b}	3.97 ^c	7.87 ^d
ERE	-0.64 ^a	0.30^{b}	0.39 ^b	0.37^{b}	-0.82^{a}	0.24^{b}	0.36 ^b	0.39^{b}	-0.34^{a}	0.22^{bc}	0.28°	0.34 ^c	-0.28^{a}	0.13 ^a	0.27^{bc}	0.35 ^c
Carcass Composition [†]																
Moisture (%)	73.3 ^c	71.1 ^b	69.7 ^{ab}	69.7 ^{ab}	71.3 ^b	69.8 ^{ab}	69.6 ^{ab}	68.9 ^a	73.2 ^e	71.3 ^{cd}	70.3 ^{bc}	69.6 ^{ab}	72.0 ^{de}	70.7^{bcd}	68.8 ^a	68.3 ^a
Protein (%)	18.6 ^a	19.2 ^{ab}	19.5 ^{ab}	19.6 ^{ab}	19.6 ^b	20.1 ^b	19.7 ^b	20.0^{b}	19.3 ^a	20.2^{ab}	20.2^{ab}	20.5^{bc}	20.4^{bc}	20.8^{bc}	21.2 ^c	20.8^{bc}
Lipid (%)	2.4 ^b	5.2 ^c	6.5 ^d	6.7 ^{de}	1.5^{a}	5.3°	6.7 ^{de}	7.4 ^e	2.4^{ab}	3.9c	5.0 ^d	5.6 ^{de}	2.0^{a}	3.3 ^{bc}	5.0 ^d	6.5 ^e
Ash (%)	6.4 ^d	5.1 ^{bc}	4.8^{ab}	4.8 ^{ab}	8.2 ^e	5.5°	4.7 ^{ab}	4.3 ^a	5.9 ^{cd}	5.4 ^{ab}	5.3 ^{ab}	5.1 ^a	6.4 ^d	5.9 ^{cd}	5.6 ^{bc}	5.1 ^{ab}
Energy (MJ kg ⁻¹)	5.04 ^a	6.38 ^b	7.00 ^{cd}	6.96 ^{bcd}	4.97 ^a	6.57 ^{bc}	6.99 ^{cd}	7.35 ^d	5.13 ^a	6.02 ^{bc}	6.46 ^{cd}	6.76 ^{de}	5.33 ^a	5.93 ^b	6.54 ^{de}	6.92 ^e

[†] Initial carcass composition (small, large). Moisture; 72.3, 71.6. Protein (fixed value); 19.1, 19.1. Lipid; 5.5, 4.0. Ash; 3.6, 5.5. Energy; 6.48, 5.86



Effect of digestible protein intake (g kg^{-0.7} day⁻¹) on PRE at 20°C (dashed line) and 26°C (solid line).



FIGURE 2

Effect of digestible energy intake (kJ kg^{-0.8} day⁻¹) on ERE at 20°C (dashed line) and 26°C (solid line).



Effect of digestible protein intake (g DP kg^{-0.7} day⁻¹) on protein gain (g kg^{-0.7} day⁻¹).



FIGURE 4

Effect of digestible energy intake (kJ kg^{-0.8} day⁻¹) on energy gain (kJ kg^{-0.8} day⁻¹). Dashed lines = 20° C; Solid line = 26° C



Partial retained energy as protein (PE; solid line) or lipid (LE; dashed line) as a function of increasing DE intake at 20 and 26°C.

4.7 The interactive effects of dietary protein and energy on feed intake, growth and protein utilization of juvenile mulloway

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ABSTRACT

The objectives of this study were to describe the interactive effects of varying digestible protein (DP) and digestible energy (DE) content on the feed intake, growth, protein utilization and whole body composition of juvenile mulloway (*Argyrosomus japonicus*) and to determine the optimal DP:DE ratio for growth. This was achieved by feeding mulloway diets containing one of four different DP levels (250 - 550 g kg⁻¹) at two DE levels (16 or 21 MJ kg⁻¹). Juvenile mulloway were stocked at each of two different sizes (70 or 200 g) in triplicate groups for each dietary treatment and fed twice daily to apparent satiation over 58 days. The results indicated that feed intake was not governed solely by energy demands but was also dependant on the DP content of the diet. Protein utilization did not improve with diets containing decreasing protein and increasing lipid content indicating that mulloway have a limited capacity to spare dietary protein. Optimal DP content was found to be 444-491 g kg⁻¹ depending on the DE content of the diet and the size of mulloway and is within the range reported for other sciaenid species. The use of formulated diets with 28.6 g DP MJ DE⁻¹ will achieve optimal growth and protein deposition for 70 – 275g mulloway.

1. INTRODUCTION

Mulloway, *Argyrosomus japonicus* (Pisces: Sciaenidae), are a euryhaline, gregarious, fast growing and highly fecund species that are easily reproduced in captivity. Mulloway have a wide distribution covering the east, western and southern seaboards of Australia (Silberschneider & Gray, 2008) and can be grown successfully in different culture systems including sea cages, ponds and recirculating aquaculture systems (Quartararo, 1996; Fielder et al., 1999; O'Sullivan & Ryan, 2001; Doroudi et al., 2006). Aquaculture of mulloway is relatively new in Australia beginning in the mid 1990's (Gooley et al., 2000). As such the industry is in its relative infancy although there has been a steady increase in production in recent years. Production of mulloway in Australia for 2004/05 was 558.4 t (O'Sullivan et al., 2007), up from 6.8 t in 1997/98 (O'Sullivan & Roberts, 2000). Development of the industry is currently restricted by a lack of knowledge of the nutritional requirements of mulloway. To date there is no published information on the requirements for digestible protein (DP) and digestible energy (DE) for mulloway and, as a consequence, no specific diet formulations are available. As a carnivorous species it is expected that mulloway will have a high requirement for DP and this is reflected in the current practice by industry of feeding mulloway commercial diets formulated for other carnivorous species such as barramundi (*Lates calcarifer*) or more generic 'marine fish' formulations.

Aquaculture feeds are formulated to maximize nutrient retention and minimize nutrient loss. This strategy is driven by both economic and environmental considerations. Nutrient utilization efficiencies have been shown to be influenced by many different factors such as species effects (Azevedo et al., 2004; Refstie et al., 2000), fish size (Einen & Roem, 1997; Azevedo et al., 2004), temperature (Bendiksen et al., 2003; Moreira et al., 2008) and the DP:DE ratio of the diet (Lupatsch et al., 2001; Booth et al., 2007). Considerable advances have been made in improving protein retention in Atlantic salmon (*Salmo salar*)

by increasing the energy content of the diet with lipid levels sometimes in excess of 30% (e.g. Einen & Roem, 1997; Hemre & Sandnes, 2008). The improved efficiencies are a result of the sparing of dietary protein from catabolism for energy by incorporating sufficient non-protein dietary energy from lipid or carbohydrate. There are, however, many examples of carnivorous marine fish such as grouper (*Epinephelus coioides*) (Luo et al., 2005), cobia (*Rachycentron canadum*) (Chou et al., 2001) and the large yellow croaker (*Pseudosciaena crocea*) (Duan et al., 2001) that show a much lower tolerance to elevated levels of dietary lipid thereby limiting potential protein sparing effects. Diets formulated with excess energy may also promote excessive lipid deposition (Shearer, 1994) and reduce feed intake (Marais & Kissil, 1979). Supplying formulated feeds with the optimal DP:DE content appropriate to a particular species, size and culture conditions is therefore crucial in maximizing nutrient retention.

The objectives of this study are to i) describe the interactive effects of varying DP and DE content on feed intake, growth, protein utilization and whole body composition of juvenile mulloway, ii) determine the optimal DP content for juvenile mulloway fed fishmeal based diets and iii) to determine the optimal DP:DE ratio for growth.

2. MATERIALS AND METHODS

2.1 Experiment design and system

The effects of varying the DP and DE content on growth and protein retention efficiency of mulloway was tested by feeding fish one of eight different diets formulated with a DP:DE ratio ranging from approximately 12 - 35 g DP MJ⁻¹ to each of two size treatments from different cohorts stocked at 10 (large; initial body weight (ibw mean \pm SD) = 199.5 \pm 11.6 g) or 20 (small; ibw = 68.7 \pm 8.5 g) fish tank⁻¹. Mulloway are a gregarious species and stocking densities were chosen to optimize growth potential (Pirozzi et al., 2009). There was no significant difference between initial weights within size treatments (p>0.5). The experiment was run over 58 days using fish produced at the New South Wales Department of Primary Industries, Port Stephens Fisheries Institute (PSFI). The experimental system consisted of two integrated 1700 l recirculating bio-filtration units supplying 48 x 200 l replicate tanks (total volume \approx 13000 l). The temperature was held at 26±0.5°C which is known to promote good growth rates in mulloway (Pirozzi, Booth, Allan unpublished data 2008) and is within the preferred temperature range for this species (Bernatzeder & Britz, 2007). Flow to each tank was approximately 4 1 min⁻¹ and orientated to create a weak centripetal current which allowed the retention of feed pellets in the tank while removing feces via a central upright 32mm diameter pvc overflow pipe which was fixed approximately 1cm off the bottom of each tank. Black plastic sheets were placed around each tank and across the top front half to minimise disturbance. Each size and feed treatment were randomly assigned to triplicate tanks with each tank constituting an experimental unit. All tanks were exposed to indirect natural light (photoperiod 12L:12D). Ammonium (NH_4^+) (<0.1 mg l⁻¹), dissolved oxygen (>5.0 mg l⁻¹), pH (7.5 - 7.9) and salinity (28 - 33 ppt) were monitored regularly throughout the duration of the experiment.

2.2 Feeds and feeding

Feeds were formulated using the linear method in Winfeed 2.8 (Winfeed Ltd., Cambrdige, UK). Each diet was formulated with either a low DE (16 MJ kg⁻¹) (LE) or high DE (21 MJ kg⁻¹) (HE) content. Digestibility coefficients of the ingredients were identified in a previous study by Booth (unpublished data, 2008). Dry ingredients were mixed in a Hobart mixer (Troy Proprietary Ltd, Ohio, USA) to make the four base diets. Each summit/diluent pair was then blended to give four different DP levels while maintaining the respective DE content giving eight diets in total (Table 1). The dry ingredients were then combined with distilled water before being pelleted through a mincer (Barnco Australia Proprietary Ltd, Leichhardt, NSW, Australia) with a 10 mm diameter die and cut to 6mm lengths. Pellets were dried in

convection drier at < 35 °C for about 6 h. Fish were fed to apparent satiation twice daily and any uneaten pellets were counted then siphoned from tanks approximately 45 min after initial feeding. Total daily feed intake was then adjusted to account for uneaten feed using the average weight derived from a sub-sample of feed pellets (n = 150 pellets for each diet) (Table 1). Fish were fasted for 48 h prior to final sampling.

2.3 Sample preparation and analyses

Initial representative samples of 10 fish of each size class were collected before the start of the experiment, euthanized with an overdose of benzocaine (ethyl-*p*-aminobenzoate) and frozen (-20°C). At the conclusion of the feeding trial all fish were euthanized, weighed and stored frozen for compositional analyses. Compositional changes were estimated by comparing the initial fish samples with those from the feeding trial. All values and subsequent reference to the nutrient and energy composition of mulloway in this study are based on whole body composition. Whole body composition was determined by placing the weighed fish into 5 l glass beakers, covering with aluminum foil and then autoclaving for 99 min at 121°C. After cooling to room temperature any changes in weight were accounted for and assumed to be changes in moisture content. The samples were then homogenised *in situ* with a hand blender and a sample taken for dry matter determination. A portion of the remaining homogenate was then transferred to plates and oven dried at approximately 80°C. The desiccated samples were then finely ground in a laboratory blender and analysed in accordance with AOAC (2005). Protein was calculated from total nitrogen based on N x 6.25 using the Dumas method. Dry matter was calculated gravimetrically after oven drying at 105°C. Ash was calculated gravimetrically after chloroform-methanol extraction.

2.4 *Performance indices*

Mass-specific data are expressed as the geometric mean of initial and final body weights of fish (GMBW) and scaled using the metabolic body weight exponent values of 0.7 for protein retention data and 0.8 for energy retention data (after Lupatsch et al., 1998; Brett & Groves, 1979). The following performance indices were calculated for each treatment group:

Daily weight gain (g fish⁻¹ day⁻¹) = Final body weight – initial body weight / number of days

Daily protein gain (g fish⁻¹ day⁻¹) = Final whole body protein content – initial whole body protein content / number of days

Daily energy gain $(kJ \text{ fish}^{-1} \text{ day}^{-1}) = Final whole body energy content – initial whole body energy content / number of days$

Daily lipid gain (g fish⁻¹ day⁻¹) = Final whole body lipid content – initial whole body lipid content / number of days

Feeding Efficiency (FE) = Weight gain / Total feed intake

Protein Retention Efficiency (PRE) = Protein gain / Total protein intake

Relative Feed Intake (RFI) (g kg^{-0.7} day⁻¹) = Total feed intake / $(GMBW/1000)^{0.7}$ / number of days

2.5 Data analyses

The effects of varying DE content (fixed; 2 levels), DP content (fixed; 4 levels) and mulloway size (fixed; 2 levels) on RFI, FE and PRE were tested using a 3-way ANOVA. Size directly influences the nutrient and energy composition of fish (Shearer, 1994); therefore data were pooled across the size term and the effects of diet on whole body composition tested as a 2-way ANCOVA with final body weight (fbw) as the co-variate. Normality of the data were checked with skewness, kurtosis and omnibus normality tests. Assumptions of homogeneity of variances were tested using modified Levenes' equal variance test. Tukey-Kramer test was used for *a posteriori* multiple comparison of means on significant terms. All results were regarded as significant at p<0.05 except for the compositional analyses where α was set at 0.01 as differences between means of <0.5% were detected when $\alpha = 0.05$. All analyses were carried out using untransformed data unless otherwise stated.

Data for PRE were non-normally distributed due to a single outlier for small mulloway fed the HE1 diet (PRE = -0.49). PRE variances were homogeneous. PRE data could not be normalized; however, ANOVA results were significant regardless of the inclusion of the outlier or not. ANOVA data are given inclusive of the outlier and due consideration should therefore be given to interpretations of the subsequent multiple comparison tests.

Water flow and air failed to one tank during the experiment resulting in the loss of all fish in that tank (large fish; HE4 diet). Survival in all the remaining tanks was 100%. Statistical analysis was completed with the mean of the remaining set substituted and degrees of freedom adjusted accordingly (Underwood, 1997).

Nonlinear regression was applied to the PRE values to determine the DP requirements of mulloway for each energy level. The asymptote of the quadratic function was considered as the optimal DP content giving the maximum PRE value.

Correlations (r) were determined between performance indices (FE and PRE) and dietary nutrients (protein, lipid and starch). Protein gain (g kg^{-0.7} day⁻¹) was also correlated with the DP:DE of the diets.

The optimal DP:DE ratio for protein gain (g kg^{-0.7} day⁻¹) was predicted using a biphasic linear model based on Koops & Grossman (1993) where protein gain (g kg^{-0.7} day⁻¹) was described as:

$$A - BSln(1 + exp^{(C-x)/s})$$

Where A = asymptote (second phase); B = linear slope (first phase); S = transition smoothness (0.5); C = transition point.

3. **RESULTS**

3.1 Diet and fish size interactions on feed intake, FE and PRE

The effects of varying DE and DP content on RFI (g kg^{-0.7}) were not dependant on the size of mulloway (i.e. there was no second-order interaction) (Table 2) however; the effect of DE on RFI (g kg^{-0.7}) varied significantly depending on the DP content of the diet. The influence of DE as well as DP on RFI also varied significantly depending on the size of mulloway (Table 2; Figure 1).

Relative feed intake $(g kg^{-0.7})$ was higher for the LE diets than the HE diets for both fish sizes and the DE x DP interaction occurred because significantly more of the LE1 diet was consumed. The DP x size

interaction occurred because, in relative terms, small mulloway consumed less of the HE1 and HE2 diets. Large mulloway consumed a proportionally greater amount of the LE diet over the HE diet hence the DE x size interaction.

The effect of dietary DE on total FE and PRE varied significantly with DP content and these interactions were different for each size class (Table 2; Figure 1). The highest FE values (mean \pm se) were 0.93 \pm 0.05 and 0.75 \pm 0.02 for small and large mulloway respectively fed the HE4 diets (Table 3; Figure 1). FE generally improved with increasing DP content with the HE diet but began to plateau from the LE2 and LE3 diets for small and large mulloway respectively.

The highest PRE values (mean \pm se) were 0.32 \pm 0.01 and 0.35 \pm 0.02 for small and large mulloway fed diets LE2 and LE3 respectively. PRE began to plateau from DP level 2 regardless of the DE content (Table 3; Figure 1). The second-order interaction for both FE and PRE occurred because of a relative improvement in efficiencies for the HE2 diet by large mulloway (Figure 1).

Correlation responses to dietary protein (r = 0.86 & 0.87; p < 0.001) and dietary starch (r = -0.84 & -0.84; p < 0.001) with respect to FE were found to be virtually identical between small and large mulloway respectively. Dietary protein (r = 0.66 & 0.65; p < 0.001) and dietary starch (r = -0.62 & -0.62; p < 0.001) were also correlated with PRE and virtually identical between the small and large mulloway (Figure 2). Dietary lipid (r = -0.73; p < 0.001 & -0.60; p < 0.01) also demonstrated a negative correlation with FE for both small and large mulloway as did PRE (r = -0.62; p < 0.001 & -0.60; p < 0.001 & -0.60; p < 0.01). The HE1 diet was found to have a large influence on the correlation coefficients with respect to dietary lipid which could be expected at a crude lipid inclusion level of 33%. With this dietary treatment level removed clear differences were found between the size classes. FE and PRE were negatively correlated with dietary lipid for small mulloway (r = -0.48, p < 0.05 & -0.62; p < 0.001); however, there was no significant correlation with large mulloway (r = -0.17, p > 0.1; -0.04, p > 0.5).

Protein gain (g kg^{-0.7} day⁻¹) was highly correlated with the DP:DE ratio of the diet (r = 0.84; p < 0.001).

3.2 Effect of dietary DE and DP on carcass composition

The effect of DE content on the moisture, lipid and energy composition of mulloway varied significantly with DP content (Table 4; Figure 2). The interaction occurred because of the relative low lipid and energy content and high moisture composition for mulloway fed the HE1 diet (Figure 2). The effect of DE on protein composition was independent of the DP content. However, both DP and DE were found to effect the protein composition of mulloway (Table 4). There was a trend for protein composition to be greater for mulloway fed LE diets and to increase with increasing DP although protein composition tended to plateau from the HE3 diets (Figure 2). The overall difference between the effect of diets on the protein composition of mulloway, although statistically significant, were quite small at less than 1% for across DE or DP content.

FBW was a significant co-variate for protein and lipid composition although, on average, the differences were small; large mulloway had only 1% greater protein and 1.2% less lipid composition than small mulloway. There was no significant effect of DE or protein on ash composition at α =0.01 (Table 4).

Estimates of optimal DP content based on maximum predicted PRE derived from the asymptotic values of 2nd order polynomial regressions were LE diet: 452 and 444 g DP kg⁻¹ and HE diet: 491 and 478 g DP kg⁻¹ for small and large mulloway respectively (Figure 3).

Figure 4 depicts the effect of DP content on protein gain (g kg^{-0.7} day⁻¹) for both LE and HE diets. This relationship can be described by the quadratic functions:

LE diet: protein gain (g kg^{-0.7} day⁻¹) = $-0.697(0.31) + 0.053(0.02)DP - 0.0005(0.0002)DP^{2}$ (±se; $r^{2} = 0.76$)

HE diet: protein gain (g kg^{-0.7} day⁻¹) = $-1.836(0.38) + 0.088(0.02)DP - 0.0008(0.0002)DP^{2}$ (±se; $r^{2} = 0.85$)

Estimates of optimal DP:DE for maximum protein gain (g fish⁻¹ day⁻¹) derived from the bi-phasic growth model were 29.8 ($r^2 = 0.86$) and 27.3 ($r^2 = 0.88$) g DP MJ DE⁻¹ for small and large fish respectively. The protein gain response to the HE1 diet (negative gain) heavily influenced the model estimates for small mulloway therefore the model was fitted excluding the HE1 data for that size. Comparison with estimates of optimal DP:DE derived from the asymptotes of quadratic functions fitted to the PRE response (HE1 data removed for small mulloway) were 29.4 ($r^2 = 0.74$) and 27.2 ($r^2 = 0.78$) for small and large mulloway respectively; these results are very similar to the estimates derived using the above bi-phasic growth model with protein gain (g fish⁻¹ da y⁻¹) as the dependent variable.

Ranges for DP:DE based on 95% CI of the fitted bi-phasic growth model overlapped for the different size classes (small, 26.6 - 33.1; large, 23.8 - 30.8 g DP MJ DE⁻¹) suggesting that a common DP:DE ratio of 28.6 g DP MJ DE⁻¹ would be suitable for formulating practical diets for mulloway of at least 70 g to approximately 275g BW (Figure 5).

4. **DISCUSSION**

It has been widely hypothesized that fish regulate feed intake to satisfy their energy requirements (Cho & Kaushik, 1990; Kaushik & Medale, 1994). The findings from the current study also support this theory however; the magnitude of the effect of DE on the relative feed intake of mulloway was shown to vary depending on the DP content of the diet. This indicates that energy requirements alone do not drive feed intake but the requirements for nutrients, in this case protein, also play a very important role.

Both the effects of DP and DE content on relative feed intake were shown to vary significantly depending on the size of mulloway. On a relative basis the demand for protein for somatic growth is known to be greater for smaller than larger fish and, conversely, the demand for energy is greater for larger than smaller fish (Lupatsch et al., 2001; Garcia-Alcazar et al., 1994). This fact is reflected in formulated diets which provide greater DP:DE content for smaller, rapidly growing fish. Large mulloway in this study consumed relatively more LE diet (compared to HE diet) than small fish presumably to meet a greater demand for metabolic energy. However, the greater overall relative feed intake demonstrated by large mulloway was likely compensatory as indicated by the initial body composition (Xie et al., 2001)

If fish also consume feed to satisfy their nutrient requirements then it would be expected that, on a relative basis, smaller fish would consume more of a low protein diet than larger fish to meet those metabolic demands for protein. However, the DP x size interaction occurred because, on a relative basis, small mulloway ate significantly less of the HE low protein diets (HE1 & HE2) compared to the higher protein diets (HE3 & HE4) than large mulloway. Indeed, small fish consuming the HE1 diet were the only group to lose weight while the corresponding feed intake for large fish was approximately equivalent to

maintenance level. This response may have occurred because high energy diets can sometimes suppress appetite (Marais & Kissil, 1979) and may indicate a shift in taste preference between size classes (Kasumyan & Doving, 2003); although this remains to be tested in mulloway. In real terms however, the difference between the amounts of HE1 & HE2 diets compared to HE3 & HE4 diets consumed by small mulloway was, on average, only 0.73 g kg^{-0.7} and would not have been significant at α .= 0.01. The different responses between small and large mulloway to diets with high DE and low DP may indicate a subtle shift in the protein:energy demands between the two sizes and highlights the importance of the correct DP:DE ratio in formulated feeds.

The effect of DE on the moisture, lipid and energy composition of mulloway was shown to vary depending on the DP content of the diet. The general trend was for lipid composition to decrease with increasing dietary protein. The exception occurred with the HE1 diet and this is concomitant with reduced feed intake at this level. Similar results have been demonstrated with silver perch (*Bidyanus bidyanus*) which show a significant linear decrease in whole body lipid composition when fed 17 MJ DE kg⁻¹ diets with increasing DP content of 173-424 g kg⁻¹; however, at 129 g DP kg⁻¹ silver perch also show a significant relative reduction in whole body lipid composition (Allan & Booth, 2004). Protein composition was shown to be affected by DE and DP content with a greater protein composition with increasing DP and decreasing DE content. A similar trend for the effect of DE on the protein composition has been reported for the cuneate drum, *Nibea miichthioides* (Wang et al., 2006); however, the influence of DP in their study, while significant, was unclear. This is possibly due to the limited range of DP (360 - 400 g kg⁻¹) used in their diets.

Mulloway in this study were found to have DP requirement of 444 - 491 g kg⁻¹ depending on the size of the fish and the dietary energy level. These values are similar to those reported for other sciaenid species such as the giant and Atlantic croakers (450 g CP kg-1; Lee et al., 2001; Davis & Arnold, 1997), the large yellow croaker (470 g CP kg-1; Duan et al., 2001) the cuneate drum (\geq 400 g DP kg⁻¹; Wang et al., 2006) and red drum (350 - 440 g DP kg-1; Daniels & Robinson, 1986; McGoogan & Gatlin, 1998).

The use of dietary protein can potentially be maximized for growth if adequate amounts of non-protein energy can be supplied to satisfy metabolic demands for energy. This protein sparing effect has been demonstrated in some fish species (e.g. Erfanullah & Jafri, 1995; Company et al., 1999; Lee et al., 2002; Azevedo et al., 2004) but not others (e.g. Peres & Oliva-Teles, 1999; De Pedro et al., 2001; Regost et al., 2001; Azevedo et al., 2004). PRE values did not significantly improve with diets containing decreasing protein and increasing lipid content; this occurred irrespective of the energy content of the diet and indicates that mulloway have a limited capacity to spare dietary protein. This effect has similarly been demonstrated in other sciaenid species (Duan et al., 2001).

Carnivorous fish are known to efficiently catabolize dietary proteins as an energy source (Gatlin III, 1995); however, whether catabolism of dietary protein by mulloway for energy is performed preferentially over non-protein energy sources (i.e. lipids) is unclear although the lack of a positive correlation between dietary lipid for both FE and PRE indicates this may be likely. Rasmussen et al. (2000) and Azevedo et al. (2004) did not observe a significant correlation between PRE and lipid intake in rainbow trout while at the same time demonstrating a significant correlation between protein intake and PRE indicating an absence of a protein sparing effect of non-protein energy nutrients. Further research is required to establish the energy utilization potential of non-protein energy sources as they pertain to mulloway.

The correct proportion of dietary DP to DE is not only important to maximize growth but imbalances have economic and environmental consequences. Diets containing excessive protein will generally be less cost effective and produce excessive nitrogenous waste (Kaushik, 1998). Diets with excessive lipid content will increase lipid deposition to the visceral cavity, liver and some muscle tissue of fish (e.g. Nanton et al.,

2007) although this may be desirable in some aquaculture species such as those consumed as sashimi (Chou et al., 2001). Protein deposition in mulloway was found to be highly correlated with the DP:DE ratio of the diet with 28.6 g DP MJ DE⁻¹ considered appropriate for 70 - 275 g mulloway. Using a factorial bioenergetic approach independent of the current study, Pirozzi, Booth & Allan (unpublished data 2008) estimated DP:DE requirements of 29.0 and 26.9 g DP MJ DE⁻¹ for 70 and 200 g mulloway respectively. These values fall well within the ranges of those established for small (26.6 - 33.1 g DP MJ DE⁻¹) and large (23.8 - 30.8 g DP MJ DE⁻¹) mulloway using the dose-response method of the current study.

While the experimental design used in this study was sensitive enough to discriminate subtle differences between the DP and DE requirements of 70 and 200 g (ibw) fish; it is important to keep in perspective that mulloway can grow up to 75 kg and 1.8 m in length (Griffiths & Heemstra, 1995). Both the sizes used in this study therefore represent rapidly growing juveniles with a high demand for dietary protein; 444 - 491 g kg⁻¹ DP depending on the fish size and energy content of the diet. Protein utilization did not improve with diets containing decreasing protein and increasing lipid content indicating that protein sparing in mulloway may be limited and underscores the importance of supplying adequate amounts of dietary protein. Protein deposition in juvenile mulloway from 70 - 275 g can be optimized with diets containing 28.6 g DP MJ DE⁻¹.

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Nutrient and ingredient profile of experimental diets (as fed basis).

		Low En	ergy Diet			High En	ergy Diet	
	LE1	LE2	LE3	LE4	HE1	HE2	HE3	HE4
Diet Ingredient (g kg ⁻¹)								
Fish Meal	344	405	435	496	191	311	372	492
Fish Oil	167	104	73	10	304	236	201	133
Blood Meal	10	103	150	243	10	66	94	150
Bovine Meal	10	10	10	10	150	138	132	120
Pregel Starch	200	152	128	80	200	152	128	80
Vit. PreMix	5	5	5	5	5	5	5	5
Diatomaceous Earth	264	221	199	156	141	92	68	20
Pellet weight (g)	0.27	0.29	0.3	0.29	0.3	0.29	0.28	0.28
Nutrient Composition								
$DM (g kg^{-1})$	951	949	947	945	949	946	944	941
Protein (g kg ⁻¹)	266	397	462	592	263	393	458	588
Fat (g kg ⁻¹)	196	142	115	61	331	27.	248	193
Ash $(g kg^{-1})$	307	274	258	225	172	140	125	93
GE (MJ/Kg)	17.12	17.30	17.44	17.65	22.50	22.54	22.58	22.66
DP (g kg ⁻¹)	257	376	436	555	251	373	434	555
DE (MJ/Kg)	15.92	15.82	15.77	15.67	20.95	20.96	20.96	20.97
DP:DE (g/MJ)	16.1	23.8	27.6	35.4	12.0	17.8	20.7	26.5

3-way ANOVA on relative feed intake (RFI), feeding efficiency (FE) and protein retention efficiency (PRE). Size = small and large mulloway (ibw ~70g and 200g); Dietary DE level = 16 and 21 MJ kg⁻¹; Dietary DP level 1 – 4 = 255, 375, 435 or 555 g DP kg⁻¹. ns = not significant at p<0.05, * = p<0.05, ** = p<0.01

			RFI			FE	PRE			
Source of										
Variation	DF	MS	F	P	MS	F	P	MS	F	Р
A: Size	1	34.33	816.30	**	0.02	6.25	*	0.01	7.79	**
B: DE Level	1	26.77	636.56	**	0.13	42.14	**	0.16	98.14	**
AB	1	0.39	9.22	**	0.08	27.81	**	0.06	36.84	**
C: DP Level	3	0.53	12.72	**	1.09	363.89	**	0.23	141.15	**
AC	3	0.16	3.73	**	0.07	24.83	**	0.04	21.38	**
BC	3	2.43	57.87	**	0.18	59.92	**	0.10	59.72	**
ABC	3	0.05	1.28	ns	0.02	6.03	**	0.02	12.64	**
Residual	31	0.04			0.00			0.00		

Summary of performance indices and carcass composition of mulloway. Fish size: Small ~70g ibw; Large ~200g ibw.

	Diet				Performa	nce Indic	es				Carcass Composition ⁵				
Fish Size	Treatment ¹	Harvest Weight	Weight ² Gain	Protein ² Gain	Energy ³ Gain	Lipid ² Gain	Feed ² Intake	RFI ⁴	FE	PRE	Moisture	Protein	Lipid	Ash	Energy
Small	Initial	-	-	-	-	-	-	-	-	-	692	184	79	48	7.25
	LE1	82.42	0.25	0.05	1.97	0.02	0.87	5.36	0.28	0.21	683	185	82	51	7.40
	LE2	96.56	0.47	0.10	2.71	0.02	0.72	4.18	0.65	0.35	699	190	66	49	6.82
	LE3	98.83	0.55	0.11	2.57	0.00	0.75	4.32	0.73	0.34	709	190	54	50	6.43
	LE4	100.73	0.56	0.13	1.90	-0.02	0.70	4.01	0.79	0.32	719	198	40	47	6.03
	HE1	65.30	-0.13	-0.04	-1.84	-0.02	0.40	2.60	-0.33	-0.37	706	173	67	57	6.48
	HE2	82.68	0.22	0.04	1.69	0.03	0.48	2.94	0.46	0.23	684	184	84	52	7.32
	HE3	88.50	0.38	0.07	2.60	0.03	0.58	3.52	0.65	0.29	692	186	76	49	7.16
	HE4	100.19	0.56	0.11	2.93	0.01	0.60	3.46	0.93	0.32	706	186	60	51	6.57
Large	Initial	-	-	-	-	-	-	-	-	-	720	190	34	61	5.59
	LE1	245.05	0.76	0.14	11.06	0.19	2.56	7.34	0.30	0.22	688	190	73	50	7.20
	LE2	263.67	1.06	0.23	9.92	0.11	2.23	6.21	0.47	0.27	702	197	51	52	6.48
	LE3	272.30	1.28	0.31	11.32	0.11	2.19	6.09	0.58	0.32	701	204	48	51	6.48
	LE4	274.99	1.28	0.32	8.30	0.02	2.11	5.83	0.61	0.27	714	205	29	53	5.83
	HE1	199.70	0.01	0.01	1.87	0.04	1.43	4.41	0.01	0.02	713	192	46	55	6.13
	HE2	251.32	0.94	0.18	12.25	0.20	1.66	4.76	0.57	0.30	687	192	72	52	7.20
	HE3	256.02	0.97	0.21	11.64	0.16	1.76	4.99	0.55	0.28	691	196	64	51	7.00
	HE4	267.47	1.21	0.27	10.94	0.11	1.61	4.51	0.75	0.30	701	198	49	54	6.50

¹ Refer to Table 1 for diet composition
² (g fish⁻¹ day⁻¹)
⁴ Relative feed intake (g kg^{-0.7} day⁻¹)
³ (kJ fish⁻¹ day⁻¹)
⁵ as received g kg⁻¹ or MJ kg⁻¹ (energy)

2-way ANOVA on protein, lipid and energy composition with final body weight (FBW) as the co-variate. Lipid data arcsine transformed. 2-way ANOVA on moisture and ash composition. ns = not significant at p<0.05, * = p<0.05, ** = p<0.01, ***= p<0.001

	Moisture Protein					Li	Α	Ash			Energy					
Source of Variation	DF	MS	F	Р	MS	F	Р	MS	F	Р	MS	F	Р	MS	F	Р
X (FBW)	1	0.01	0.01	ns	11.54	46.37	**	26.12	53.11	**	0.35	3.98	ns	0.33	5.62	*
A: DE Level	1	2.16	4.25	*	3.71	14.92	**	14.48	29.44	**	0.58	6.54	*	0.42	7.13	*
B: DP Level	3	6.19	12.2	**	2.03	8.17	**	18.47	37.55	**	0.24	2.75	ns	1.07	18.07	**
AB	3	10.56	20.78	**	0.16	0.62	ns	19.65	39.95	**	0.22	2.45	ns	1.96	33.26	**
Residual	38	0.51			0.25			0.49			0.09			0.06		



First-order interaction (DE x DP and DP x size) on relative feed intake (RFI). Second-order interaction (DE x DP x size) on feeding efficiency (FE) and protein retention efficiency (PRE). Data points are means \pm se (n = 3; n = 2 for large HE4). Solid circles = high energy diets (HE; 21 MJ kg⁻¹); Open circles = low energy diets (LE; 16 MJ kg⁻¹). Dietary DP level 1 - 4 = 255, 375, 435 or 555 g DP kg⁻¹. Small ~70g; large ~200g ibw. Tukey-Kramer test on means within the DE x DP interaction term for each size class. Diets shown ranked by mean values from highest (top) to lowest (bottom). Means sharing lines are not significantly different (p>0.05)



Effect of dietary DE on lipid, moisture, energy and protein composition of mulloway dependant on dietary DP content. Data are pooled means across size terms (\pm se; n = 6; n = 5 for HE4). Solid circles = high energy diets (HE; 21 MJ kg⁻¹); Open circles = low energy diets (LE; 16 MJ kg⁻¹). Dietary DP level 1 – 4 = 255, 375, 435 or 555 g DP kg⁻¹



Effect of dietary DP and DE on PRE. Asymptote of quadratic functions represent required dietary DP for optimal PRE. (\pm se; n = 3; n = 2 for Large HE at 555 g DP kg⁻¹). HE = 21 MJ kg⁻¹; LE = 16 MJ kg⁻¹.



Effect of dietary DP and DE on protein gain (g kg^{-0.7} day⁻¹). (\pm se; n = 6; n = 5 for HE at 55.5 g DP 100g⁻¹). HE = 21 MJ kg⁻¹; LE = 16 MJ kg⁻¹.



FIGURE 5

Effect of dietary DP:DE ratio (g DP MJ DE⁻¹) on protein gain (g kg^{-0.7} day⁻¹). (\pm se; n = 6; n = 5 for 26.5 g DP MJ DE⁻¹; HE1 data for small mulloway removed from analyses). Maximum protein deposition at 28.6 g DP MJ DE⁻¹. Dashed vertical lines represent 95% confidence limits at 26.6 - 30.8 g DP MJ DE⁻¹. HE = 21 MJ kg⁻¹; LE = 16 MJ kg⁻¹.

4.8 The routine metabolic rate of mulloway (*Argyrosomus japonicus*: Sciaenidae) and yellowtail kingfish (*Seriola lalandi*: Carangidae) acclimated to six different temperatures

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ABSTRACT

This study compared the mass-specific routine metabolic rate (RMR) of similar sized mulloway (Argyrosomus japonicus), a sedentary species, and yellowtail kingfish (Seriola lalandi), a highly active species, acclimated at one of several temperatures ranging from 10 -35° C. Respirometry was carried out in an open-top static system and RMR corrected for seawater-atmosphere O₂ exchange using mass-balance equations. For both species RMR increased linearly with increasing temperature (T). RMR for mulloway was 5.78T - 29.0 mg O_2 kg^{-0.8} h⁻¹ and for yellowtail kingfish was 12.11T -39.40 mg O_2 kg^{-0.8} h⁻¹. The factorial difference in RMR between mulloway and vellowtail kingfish ranged from 2.8 to 2.2 depending on temperature. The energetic cost of routine activity can be described as a function of temperature for mulloway as 1.93T - 9.68 kJ kg^{-0.8} day⁻¹ and for yellowtail kingfish as 4.04T - 13.14 kJ kg^{-0.8} day⁻¹. Over the full range of temperatures tested Q_{10} values were approximately 2 for both species while Q_{10} responses at each temperature increment varied considerably with mulloway and yellowtail kingfish displaying thermosensitivities indicative of each species respective niche habitat. RMR for mulloway was least thermally dependent at 28.5°C and for yellowtail kingfish at 22.8°C. Activation energies (E_a) calculated from Arrhenius plots were not significantly different between mulloway (47.6 kJ mol⁻¹) and yellowtail kingfish (44.1 kJ mol⁻¹).

Keywords: Routine metabolic rate; Metabolism; Temperature; Oxygen consumption; Open-top respirometry; Oxygen transfer

1. INTRODUCTION

Mulloway (Argyrosomus japonicus) and yellowtail kingfish (Seriola lalandi) are marine carnivores that are important food species and highly sort after sport fish. They are both important aquaculture species in Australia and are cultured in sea cage or on-shore recirculation systems. While both species sometimes naturally co-occur each occupy distinct niche habitats. Mulloway are found in estuarine, near-shore waters and surf zones (Griffiths, 1996; Griffiths, 1997; Silberschneider & Gray, 2008). Yellowtail kingfish are a schooling pelagic species with a circumglobal distribution and are found in both near-shore and off-shore waters (Kailola et al., 1993). In Australia both species are similarly distributed around the eastern and southern seaboards although yellowtail kingfish are also found in cooler temperate waters of the Bass Strait and Tasmania while mulloway are also found in the warmer temperate waters to the North West Cape of Western Australian (Kailola et al., 1993). Both species possess distinct physiological and morphological characteristics adapted to exploit their respective niche environments. Mulloway are a relatively sedentary species (Silberschneider & Gray, 2008) that are euryhaline (Fielder & Bardsley, 1999; Harrison & Whitfield, 2006), eurythermal (Harrison & Whitfield, 2006), hypoxia tolerant (Fitzgibbon et al., 2007) and have a low aerobic capacity similar to rainbow trout and Atlantic cod (Bushnell et al., 1984; Schurmann & Steffensen, 1997; Fitzgibbon et al., 2007). In contrast, yellowtail kingfish are a high-performance highenergy-demand species which share many of the specialized morphological adaptations of

the tunas (Dewar & Graham, 1994; Clark & Seymour, 2006) and have an aerobic metabolic scope similar to that of other highly active teleost species such as salmon (Clark & Seymour, 2006).

Metabolism reflects the uptake, transformation and allocation of energy and materials for maintenance, growth and reproduction; the rate at which this occurs determines the pace of an organism's life (Brown et al., 2004). Metabolic theory links metabolic rate on a broad scale to the ecology of populations and ecosystem processes (Brown et al., 2004; van der Meer, 2006) while at the organismal level life history traits such as development time (Finn et al., 2002; Gillooly et al., 2002), mortality rate (Brown et al., 2004) and reproductive rate (Savage et al., 2004) can also be predicted (Brown et al., 2004). An individual's metabolic rate is predominately a function of its mass (Schmidt-Nielsen, 1975; West et al., 2002) but will also vary considerably depending on its activity level (Boisclair and Tang, 1993), health (Barton, 1997) and nutritional status (Jobling, 1982). Temperature is one of the most important extrinsic factors influencing metabolic rate in ectotherms, directly governing the speed at which biochemical and physiological processes proceed (Clarke & Johnston, 1999; Hochachka & Somero, 2002) as well as having a direct influence on activity (Jobling, 1982). The relationship between temperature and metabolic rate is strongly linked to the temperature dependence of enzymatic reactions (Hochachka & Somero, 2002).

Respiration rate, measured in terms of oxygen consumption (MO_2), is an accurate proxy for metabolic rate (Withers, 1992). Deriving an organism's metabolic rate in this way is useful as it firstly provides a direct measure of the animals' requirement for oxygen, information which is critical for the culture of any aquatic species and secondly, it allows indirect estimations of the requirements for energy (Elliott and Davison, 1975). Metabolic rates of mulloway and yellowtail kingfish have been measured previously; however only at a single temperature for mulloway ($22^{\circ}C$; Fitzgibbon et al., 2007) and over a small temperature range for yellowtail kingfish ($20-25^{\circ}C$; Clark & Seymour, 2006). Those studies showed that the standard metabolic rate (SMR) of mulloway and yellowtail kingfish, scaled for body weight and temperature, were similar (Clark & Seymour, 2006; Fitzgibbon et al., 2007).

In this study, we examine the influence of a wide range of temperatures on the routine metabolic rate (RMR) of mulloway and yellowtail kingfish where RMR reflects the MO_2 during routine activity and spontaneous movement of unfed, but not starving fish (Fry, 1957; Beamish, 1964). The overall objectives were twofold; firstly to validate an open-top respirometry system and secondly, to describe the RMR of mulloway and kingfish as a function of temperature. As biogeographic patterns of distribution and abundance are closely linked to the temperature-adaptive physiology of ectotherms (Hochachka & Somero, 2002; Somero, 2005), we hypothesize that the RMR of mulloway and yellowtail kingfish will closely reflect those characteristics noted above; i.e. relative to yellowtail kingfish, mulloway will 1) have a lower RMR 2) show a reduced thermosensitivity response to different acclimation temperatures and 3) exhibit a higher temperature at which RMR is least thermally dependant.

2. MATERIALS AND METHODS

2.1 Respirometry validation

 MO_2 readings used to calculate the metabolic rates of mulloway and yellowtail kingfish in this study were measured in an open-top system; i.e. the surface water was exposed to the atmosphere. Therefore, O_2 transfer coefficients at each temperature treatment were established in a separate trial to account for atmospheric transfer of O_2 into seawater at sea level (1013 hPa). This was achieved by depleting DO levels down to 60% saturation using nitrogen gas and measuring the rate of increase to resaturation. Duplicate 200 l experiment tanks (dimensions: top diameter = 78 cm; bottom diameter = 68 cm; height = 55 cm) were
placed in the sumps (fiberglass tanks 2.7 x 1.2 x 0.6 m) of each recirculation system (described below) which acted as water baths maintaining constant temperatures. Inside tank surfaces were scrubbed down and disinfected with sodium hypochlorite (NaOCl) before the start of each temperature reaeration trial, rinsed and refilled with seawater which had also been treated with NaOCl and neutralized with sodium thiosulphate (Na₂S₂O₃). A small submersible aquarium pump (flow rate approximately 5 l min⁻¹) was placed at the bottom of each 200 l tank and positioned to create a small turbulent flow to mimic fish movement in the tanks; initial DO readings taken at the near-surface, middle and bottom of tanks were virtually identical indicating complete mixing. DO Readings for each temperature trial were taken at intervals of approximately 0, 1, 6, 18 and then every 24 h up to10 d or until resaturation was achieved.

2.2 RMR experiment design & fish handling

The mass-specific routine metabolic rate (RMR; mg $O_2 \text{ kg}^{-0.8} \text{ h}^{-1}$) was established at 6 temperatures for mulloway (10, 15, 20, 25, 30 or 35°C) and yellowtail kingfish (10, 15, 20, 25, 30 or 32.5°C). Fish were F1 juveniles of wild caught broodstock held at the New South Wales Department of Primary Industries, Port Stephens Fisheries Institute (PSFI).

Eight mulloway (181.8±4.4 g; mean±SD) or 5 yellowtail kingfish (206.0±7.0 g) were stocked in triplicate groups into 200 l tanks for each of the 6 temperature treatments with each tank constituting an experimental unit. The recirculation system supplying the tanks was split into two independent banks with one bank designated as cool water $(10 - 20^{\circ}C)$ while the other designated as warm water $(25 - 35^{\circ}C)$. Fish were initially stocked at ambient water temperature (23°C) then, depending on which system they were assigned, adjusted up or down to specific temperature treatments in increments of 1°C day⁻¹ to ensure complete acclimation (Mora and Maya, 2006). Fish were held for one week at that temperature before MO_2 readings were taken using a Luminescent Dissolved Oxygen (LDOTM) meter (model HQ30d-LDO101-03; Hach Company, Loveland, CO, USA) which was calibrated at each temperature treatment according to manufacturer's instructions. The temperature for each unit was controlled with a chiller and immersion heater operating in an antagonistic mode which allowed precise temperature control of ±0.5°C of the set temperature. Constant water flow (360 l h^{-1}) and air were supplied to all tanks when MO₂ was not being recorded. 100% medical grade O_2 was injected into the main water supply manifold for the high temperature treatments (30 and 35°C). After fish were initially stocked PVC tubes 300 mm long and 32 mm diameter perforated with 10 mm holes were positioned vertically down from the centre surface of each tank to act as sleeves through which the LDO probe could be introduced discretely into tanks without disturbing the fish. Black plastic sheets were also placed across the front top half of tanks to prevent disturbance to fish from the presence of workers taking MO₂ readings.

A power failure occurred over night during the acclimatization period from 33 to 34° C resulting in the loss of 37.5% of the mulloway and 100% of the yellowtail kingfish from the warm water system. Mortalities were likely due to low DO levels. There was 100% survival of both mulloway and yellowtail kingfish in the corresponding cool water system (12 to 11°C). Both species were restocked for the warm water treatment group at ambient temperatures (26°C) and re-acclimated following the above protocols. No further mortalities occurred. Some yellowtail kingfish were previously observed to regurgitate food at 33°C; therefore it was decided upon restocking to end MO_2 readings at 32.5°C for that species.

Water quality parameters were consistent between systems and pH (7.93 – 8.16), NH_4^+ (<0.1 mg l⁻¹) and salinity (33.4 – 35.0 ppt) were monitored regularly. All fish were fed a maintenance ration once daily of a commercial diet (Ridley AquaFeed Pty. Ltd., Narangba, Qld. Australia; 45.5% crude protein, 18.7% crude fat, 22.2 MJ kg⁻¹ gross energy). All fish were fasted for 48 h prior to MO_2 readings.

2.3 MO₂

 MO_2 was established by measuring the decrease of DO in standing water over approximately 1 hourly intervals. Measurements were repeated three times over several hours for each replicate tank and the mean used to calculate RMR. For all MO_2 trials DO levels were at or near saturation at start readings and always remained above 60% after 1 h. After each end reading water flow was re-established for approximately 1 h to flush tanks of metabolites and return DO to saturated levels. Each temperature treatment group was independent and fish were removed from the system and re-weighed after completion of MO_2 readings for that particular temperature and species group. Background biochemical oxygen demand (BOD) was then determined for each replicate tank after fish were removed and water re-saturated with O_2 .

2.4 Data analyses

2.4.1 Atmosphere-seawater oxygen transfer

Predicted rates of reaeration were derived by first establishing the relationship between O_2 saturation (%) and O_2 concentration (mg l⁻¹) at temperature (*T*) according to the linear relationship of the coefficients when *x* and *y*-intercepts = 0 (r^2 =0.998). Equivalent oxygen concentrations (mg l⁻¹) could then be established for Top (100%) and Bottom (60%) saturation levels at any temperature (*T*). O_2 transfer (OT; mg l⁻¹) and O_2 transfer rates (OTR; mg l⁻¹ h⁻¹), applicable to the system and conditions used in the current study, can be described by the exponential associations:

$$OT = Bottom + (Top - Bottom) \times (1 - exp(-k \times t))$$
 1.

Where Bottom = O_2 concentration (mg l⁻¹) at 60% saturation at temperature *T*; Top = O_2 concentration (mg l⁻¹) at 100% saturation at temperature *T*; *k* = rate constant; *t* = time (h)

$$OTR = (Top - Bottom) \times exp(-k \times t)) + Bottom$$
 2.

Where Top = max. OTR (mg l⁻¹ h⁻¹) at temperature T when t = 0; Bottom = 0 (saturated; fixed); k = rate constant; t = time (h)

Rate constants were described as a function of temperature according to the linear relationship of k (y-axis) and T (x-axis) for OT (when x and y-intercepts = 0; $r^2 = 0.98$) and also for OTR (y-intercept $\neq 0$; $r^2 = 0.78$). By solving for t in equation 1 at a known OT value (geometric mean of beginning and end reading) the OTR could then be derived at any point of the O₂ gradient between 60-100% saturation and for any temperature between 10- 35° C.

2.4.2 *Metabolic indices*

References to temperature (T) are in °C unless otherwise stated where temperature is absolute temperature in °K. Mass-specific data are scaled using the metabolic body weight exponent of 0.8 (Brett and Groves, 1979). Once OTR and background BOD were established the mass-specific RMR for each species at each temperature could then be calculated as:

$$RMR (mg kg^{-0.8} h^{-1}) = (V/BW/n) \times (\Delta O_2 - O_{2otr} + O_{2bod})$$
3.

Where V = tank water volume (l); BW = mean body weight (kg^{-0.8}); n = number of fish tank⁻¹; ΔO_2 = net change in O_2 concentration (mg l⁻¹ h⁻¹) inclusive of fish respiration, atmospheric re-aeration and background BOD; O_{2otr} = atmospheric OTR (mg l⁻¹ h⁻¹); O_{2bod} = background BOD rate (mg l⁻¹ h⁻¹)

Interspecific differences in the relationship between RMR and temperature were analyzed using linear regression. ANCOVA was used to compare slopes and elevations (Motulsky and Christopoulos, 2003). Factorial difference was calculated as $RMR_{yellowtail kingfish} / RMR_{mulloway}$. All results were regarded as significant at *p*<0.05. Data are presented as mean ± standard error.

Kinetic function was indexed by the effects of temperature on the apparent activation energy (E_a) of each species with E_a determined directly from the slope of Arrhenius plots using the equation (Kotz and Treichel, 1996):

$$E_a = -slope \ x \ R$$

4.

where slope = $\Delta \ln RMR / \Delta(1/K)$, K is absolute temperature (°K) and R is the universal gas constant (8.3145 x 10⁻³ J mol⁻¹ K⁻¹). Slope discontinuities in Arrhenius plots can indicate perturbations in the underlying rate process and are identified at the Arrhenius breakpoint temperature (ABT) (Hochachka & Somero, 2002). ABT's were not detected for either species i.e. respiration rates did not fall at high temperatures, however the temperature sensitivity of RMR was described by applying nonlinear regression to temperature quotient (Q_{10}) values plotted as a function of the geometric mean temperature (°C) with the asymptote describing the temperature which has the minimum influence on RMR with respect to a 10°C shift in temperature. Q_{10} values were established at each temperature interval using the equation:

$$Q_{10} = (M_2 / M_1)^{10/(T_2 - T_1)}$$

5.

Where M_1 and M_2 are the RMR values at temperatures T_1 and T_2 respectively.

3. **RESULTS**

3.1 Respirometer validation

Table 1 describes the theoretical parameters applicable to the system and conditions used in the current study to estimate reaeration rates in equations 1 and 2.

Background BOD rates at low temperatures (10 and 15°C) were typically beyond the resolution limits of the LDO probe to detect a change (i.e. <0.01 mg l⁻¹) and were therefore assumed to equal that of the theoretical OTR applicable to that temperature. Background BOD generally increased with increasing temperature with BOD in yellowtail kingfish tanks tending to be higher in warmer water than mulloway tanks (Figure 1). BOD slopes between species were significantly different (p<0.05). Average (OTR adjusted) background BOD ranges were approximately 0.005-0.030 (mgO₂ l⁻¹ h⁻¹) depending on temperature and species tank.

There were no significant differences found between the RMR of uncorrected data when compared to values that were corrected for OTR and background BOD (p>0.5; Figure 2). The proportion of ΔO_2 attributed to fish respiration far exceed that due to OTR and background BOD; ranging from the lowest at 97.1±0.1% for mulloway tanks at 35°C to 99.9±0.1% for yellowtail kingfish tanks at 10°C.

3.2 Metabolism

The following results are reported as corrected data. RMR of both mulloway and yellowtail kingfish was shown to increase significantly with temperature (Figure 2). RMR was significantly different (p<0001) between species at each temperature. RMR for mulloway ranged from 33.0±0.6 mg O₂ kg^{-0.8} h⁻¹ at 10°C to 180.2±11.7 mg O₂ kg^{-0.8} h⁻¹ at 35°C while the RMR of yellowtail kingfish ranged from 85.3±4.5 mg O₂ kg^{-0.8} h⁻¹ at 10°C to 382.3±8.9 mg O₂ kg^{-0.8} h⁻¹ at 32.5°C. The relationship between temperature and RMR was linear for both species (Figure 2) and can be described as:

RMR
mulloway = 5.783T - 29.004 $(r^2 = 0.97)$ 6.RMR
vellowtail kingfish = 12.113T - 39.402 $(r^2 = 0.95)$ 7.

The factorial difference between RMR of mulloway and yellowtail kingfish derived from equations 6 and 7 was not constant but decreased exponentially with increasing temperature:

RMR factorial difference = $3.469 \times \exp(-0.174T) + 2.222$ ($r^2 = 0.99$) 8.

There was no significant difference between the slopes of Arrhenius plots for mulloway and yellowtail kingfish (p>0.25; Figure 3). The Arrhenius relationship can be described as:

 $\ln RMR_{mulloway} = -5.729(1/Kx10^3) + 23.876 \qquad (r^2=0.97) \qquad 9. \\ \ln RMR_{yellowtail kingfish} = -5.320(1/Kx10^3) + 23.360 \qquad (r^2=0.95) \qquad 10.$

 E_a can be calculated as 47.6(±2.1) and 44.1(±2.8) kJ mol⁻¹ for mulloway and yellowtail kingfish respectively.

The lowest Q_{10} for mulloway ($Q_{10} = 1.5$) occurred between 25-30°C and for yellowtail kingfish ($Q_{10} = 1.2$) between 20-25°C (Figure 4). Q_{10} values were similar for both species over the entire temperature range ($Q_{10 (10-35)} = 2.0$ for mulloway and $Q_{10 (10-32.5)} = 1.9$ for yellowtail kingfish). The relationship between Q_{10} and temperature can be described as:

$Q_{10\text{mulloway}} = 5.797 - 0.297T + 0.005T^2$	$(r^2 = 0.99)$	11.
$Q_{10\text{yellowtail kingfish}} = 7.805 - 0.5489T + 0.012T^2$	$(r^2 = 0.65)$	12.

The asymptotes of equations 10 and 11 occurred at 28.5 and 22.8°C for mulloway and yellowtail kingfish respectively.

Using the oxyenergetic coefficient of 13.59 kJ mg⁻¹ O_2 (Elliott and Davison, 1975) the daily energetic cost of post-absorptive routine activity can be described as a function of temperature as:

mulloway (kJ kg ^{-0.8} day ⁻¹) = $1.929T$ -	- 9.677	$(r^2=0.97)$	13.
yellowtail kingfish $(kJ kg^{-0.8} day^{-1}) =$	4.041 <i>T</i> – 13.141	$(r^2=0.95)$	14.

4. **DISCUSSION**

By nature of the gaseous phase, respirometers designed to estimate metabolism in air breathing terrestrial animals require air-tight chambers to account for O2 and CO2 budgets (e.g. Misson, 1974; Moors, 1977). This study demonstrated that the air-water interface in a static system provides a sufficient boundary layer to establish reliable metabolic estimates and, although this boundary layer is permeable, MO_2 through fish respiration can easily be accounted for through simple mass-balance equations. Re-aeration and background BOD rates were shown to have a numerically small but statistically insignificant influence on the measured RMR of mulloway and vellowtail kingfish. This was because the proportional contribution of fish respiration to the change in DO concentration at all temperatures far exceeded that due to re-aeration and background BOD. The advantages of establishing MO_2 in an open system such as the one used in this study are many. MO_2 of groups of fish is less likely to be confounded by elevated stress which may occur with individually housed fish, particularly with gregarious species such as mulloway. Acclimation periods can easily be of a sufficient duration to ensure relatively normal stress levels. Logistically, the open-top system is much more practical and cost effective than traditional respirometers and, as such, allows for greater replication and experimental power. Contiguous measurements over longer periods in a static system can be achieved by intermittent flow to avoid hypoxic conditions (Forstner, 1983; Kaufman et al., 1989).

RMR is a useful index of metabolic requirement as most fish maintain routine swimming velocities. The RMR of similar size mulloway and yellowtail kingfish was shown to be linearly dependant on water temperature while comparisons between the species clearly demonstrated a greater demand for oxygen by yellowtail kingfish; the RMR of both species being equivalent when yellowtail kingfish are at temperatures approximately half that of mulloway. This difference was made evident during the power failure at 33-34°C when 100% of the yellowtail kingfish were lost while 62.5% of mulloway survived. The high oxygen demand of yellowtail kingfish has clear implications for the aquaculture of this species. While mulloway are known to be relatively hypoxia tolerant (Fitzgibbon et al., 2007) it is critical that high DO levels are maintained for yellowtail kingfish, preferably at saturated levels.

During routine activity yellowtail kingfish will consume, depending on temperature, approximately two to three times as much oxygen as mulloway and will therefore require two to three times as much energy intake to fuel routine metabolism. In a feeding study by Pirozzi et al. (2010) carried out using the same recirculation system and experiment tanks as the current study, the daily maintenance digestible energy (DE) requirements for mulloway were found to be 44.2 and 49.6 kJ DE kg^{-0.8} day⁻¹ at 20 and 26°C respectively. These values are higher than the energy requirements estimated from equation 13 (cf. 28.9 and 40.5 kJ kg^{-0.8} day⁻¹ at 20 and 26°C respectively). Maintenance energy requirements derived from feeding studies in this way are inclusive of the increased energetic costs associated with prandial metabolism including specific dynamic action (SDA) as well as general feeding activity. Clearly the maintenance energy requirements of fasted fish at routine swimming velocities compared to that of actively feeding fish represent different levels within the metabolic scope of activity. This has important implications for the construction of bioenergetic models used to make predictions of energy requirements, which in turn provide the foundation for diet formulations and feeding strategies for cultured fish (Bureau et al., 2002). A clear delineation between the different levels of activity must be made to ensure the integrity of models predicting "maintenance" energy requirements.

Fish have a lower metabolism and a correspondingly lower activity level at colder temperatures (Jobling, 1982; Fonds et al., 1992). While swimming velocities were not recorded in this study, the stark contrast in activity exhibited by the two species is likely to have contributed towards the significant differences seen in overall *MO*₂; mulloway activity

was generally restricted to maintaining their position at the bottom of the tank while yellowtail kingfish continued to swim actively throughout the water column, although this activity was quite noticeably reduced in cold temperatures. Normalized for temperature and size, the standard metabolic rates (SMR) of mulloway and yellowtail kingfish have been shown to be similar although the aerobic scope of yellowtail kingfish is almost 3 times that of mulloway (Clark & Seymour, 2006; Fitzgibbon et al., 2007). The decrease in the factorial difference in MO_2 with increasing temperatures between mulloway and yellowtail kingfish may give some insight into the thermoregulatory responses between the two species. The relative difference between standard and routine metabolism in some species has been shown to decrease with increasing temperature (Hölker, 2003) and this may be because cold temperatures are known to enhance the oxidative capacity of skeletal muscle and other tissues (Guderley & Johnston, 1996).

The RMR of mulloway at 22°C (98 mg kg^{-0.8} h⁻¹), derived from equation 6, is very close to the MO_2 at the slowest swimming velocity recorded by Fitzgibbon et al. (2007) for mulloway at this temperature in normoxic conditions using a tunnel respirometer. Fitzgibbon et al. (2007) point out that MO_2 of mulloway at the slowest test velocity (7.5 cm s⁻¹) was slightly higher than that observed at 15 cm s⁻¹ (86 mg kg^{-0.8} h⁻¹) and attribute this to the energetic cost associated with maintaining stability at low velocities. Similar responses in MO_2 as a function of low swimming velocity have also been recorded for the European seabass (*Dicentrarchus labrax*) (Chatelier et al., 2005) and Pacific bonito (*Sarda chiliensi*) (Sepulveda et al., 2003). While estimations of RMR, by definition, include MO_2 associated with spontaneous activity (Fry, 1957), estimates of SMR derived by extrapolating relative swimming speed to 0 velocities can be influenced by the energetic cost of stability at low swimming velocities (Magnuson, 1973; Webb, 2002) particularly in obligate ramventilating species such as the tunas and sharks (Sepulveda et al., 2003; Sepulveda et al., 2007).

While yellowtail kingfish are a high-energy-demand, high-performance species that share similar morphological characteristics of the tunas (Clark & Seymour, 2006) the RMR of tuna species is much higher. Southern bluefin tuna (*Thunnus maccoyii*), a species that are also cultured in Australia, have a RMR of 834 mg kg^{-0.8} h⁻¹ at 19°C (Fitzgibbon et al., 2008) which is more than four times that of yellowtail kingfish and more than ten times than that of mulloway at the same temperature (*cf.* 191 and 81 mg kg^{-0.8} h⁻¹ for yellowtail kingfish and mulloway respectively). The high metabolic rate of tunas is related in part to their elevated endothermy and obligate ram-ventilating requirement (Sepulveda et al., 2003); characteristics which are absent in other teleosts such as mulloway and yellowtail kingfish.

From equation 6 the predicted RMR of mulloway at 26°C (121 mg O_2 kg^{-0.8} h⁻¹) is slightly higher than that of the similarly sedentary barramundi (*Lates calcarifer*) held between 26-32°C (92 mg O2 kg^{-0.8} h⁻¹) (data adapted from Glencross and Felsing (2006) based on a 180 g fish). Barramundi are a catadromous species that are also cultured in Australia, and while the study by Glencross and Felsing (2006) was carried out on barramundi in freshwater, the energetic cost associated with osmoregulation by euryhaline species such as mulloway in seawater may not necessarily equate to a significant relative increase in MO_2 . The influence of salinity on MO_2 , and therefore energy metabolism, varies considerably depending on the species and rearing conditions (Claireaux and Lagardere, 1999; Altinok and Grizzle, 2003; Wuenschel et al., 2005) as well as life history (Morgan & Iwama, 1991) making generalizations very difficult and necessitating the establishment of MO_2 and salinity relationships for each species as required.

The thermosensitivity of RMR in yellowtail kingfish demonstrated a clear parabolic response with the lowest Q_{10} occurring between 20-25°C and the asymptote at 22.8°C. While not strictly stenotherms, the increased thermosensitivity of RMR outside these ranges indicate that yellowtail kingfish have a narrower temperature range for optimal metabolic

The $Q_{10(20-25)}$ value of 1.2 for yellowtail kingfish recorded in this study is considerably lower than that reported by Clark & Seymour (2006) on the SMR of the same species $(Q_{10(20-25)} = 4.5; BW = 2.1 \text{ kg})$. The short acclimation period, although noted as ecologically relevant in their study, of 5°C over 3 h (cf. 2-3°C in 10 d this study) is likely the main reason for such discrepancy and highlights the need for acclimation periods of adequate duration if acute temperature related responses in MO₂ are not desired. The thermosensitivity of RMR in mulloway demonstrated a reverse J-curve response with the lowest Q_{10} occurring between 25-30°C and the asymptote at 28.5°C. In contrast to yellowtail kingfish, the curve relating to Q_{10} values between 20-35°C was very shallow showing little difference over this range indicating that mulloway have a much broader ranging thermal tolerance on metabolic function, typical of eurythermal species inhabiting estuarine and near-shore coastal habitats (Harrison & Whitfield, 2006). The thermosensitivity of RMR has implications for the aquaculture of both species particularly in terms of seasonal temperature profiles at site locations for sea cage operations. Temperatures consistently above or below those least thermally dependent ranges may have negative impacts on productivity.

Ectotherms exhibit thermoregulatory behaviour by altering spatial and temporal patterns of activity to maintain their body temperature within a narrow "optimal" range (Beitinger & Fitzpatrick, 1979; Hochachka & Somero, 2002). This is linked with the idea that final thermal preferenda and thermal physiology are closely co-adapted and that thermal preferences coincide with temperatures that maximize Darwinian fitness (Beitinger and Fitzpatrick, 1979; Angilletta et al., 2006; Martin and Huey, 2008). Martin and Huey (2008) however proposed the concept of "suboptimal is optimal" in ectotherms whereby the preferred temperature may be lower than the physiologically optimal temperature. Their model predicts that animals will select temperatures that are somewhat lower than the temperature at which fitness is maximal (Martin and Huey, 2008). Thermal studies on mulloway by Bernatzeder & Britz (2007) demonstrated a final preferred temperature range of 25 – 26.4°C while the predicted temperature in the current study at which the RMR of mulloway was the least thermally dependent was somewhat higher at 28.5°C. The Martin and Huey model (2008) may apply in this case only if we consider 28.5°C near the physiologically "optimal" temperature for mulloway. We may then speculate that yellowtail kingfish will select temperatures slightly below 22.8°C if given a choice; however this remains to be tested. It should be remembered however that the RMR values established in this study are of the routine activity of post-absorptive juvenile fish and therefore exclude the influence of post-prandial effects and specific dynamic action (SDA); thermal sensitivities may shift slightly depending on physiological (and reproductive) states (Angilletta et al., 2002). Indeed, studies on the thermal effects of post-prandial metabolic responses in juvenile mulloway (Pirozzi and Booth, unpublished data, 2008) show a shift in Q_{10} values of approximately 0.2 when comparing the metabolic thermosensitivities between peak SDA MO₂ and RMR of 240 g fish. Furthermore a change in the direction of thermosensitivity ($Q_{10peakSDA}$ - Q_{10RMR}) was seen depending on the shift in temperature from -0.2 $(Q_{10(14-20)})$ to +0.2 $(Q_{10(20-26)})$.

As the body temperature of ectotherms conforms to the temperature of their immediate environment it is therefore reasonable to consider that their metabolism also responds in the same way of simple chemical reactions. According to kinetic theory chemical reactions only proceed once they have attained a minimum required energy of activation (E_a). Generally, at around room temperature (25°C) reaction rates with an E_a of ~50 kJ mol⁻¹ double for every 10°C rise in temperature (Kotz & Treichel, 1996). Over the range of temperatures tested in this study the E_a values of mulloway (47.6 kJ mol⁻¹) and yellowtail kingfish (44.1 kJ mol⁻¹) were found to be very close to this value and generally conform to the overall Q_{10} for most fish species (Cameron, 1989; Clarke & Johnston, 1999). For similar reactions at a given temperature the greater the energy barrier (i.e. the higher the E_a) the slower the reaction rate (Kotz and Treichel, 1996); we can therefore expect reaction rates within mulloway to proceed at a similar rate as yellowtail kingfish. The difference in routine activity between mulloway and yellowtail kingfish appeared to influence the elevation but not the slope (Figure 4) indicating that E_a may be independent of activity level. Similar differences between the elevation but not the slope of Arrhenius plots can also be seen between the SMR and RMR of carp (*Cyprinus carpio*) (Becker et al., 1992).

To conclude, re-aeration rates and BOD levels of seawater in the open-top system used in this study were shown to have an insignificant influence on estimations of RMR; even so, metabolic rates can be accurately quantified using simple mass balance equations to account for minor influences not directly associated with fish respiration. Comparable results to published data on the same species using more traditional flow-through respirometers also lend confidence to the system and methods used. The thermosensitivity response of RMR appeared indicative of the temperature profiles where mulloway and yellowtail kingfish are naturally found. This has direct implications for the aquaculture of the species particularly with regard to appropriate site locations with exposure to optimal temperature ranges; i.e. 20-25°C for yellowtail kingfish and 25-30°C for mulloway, although mulloway should still perform well if temperatures remain above 20°C. The high oxygen demand of yellowtail kingfish necessitates the supply of high levels of DO in any culture system for this species.

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Parameter values used to populate equations 1 and 2 describing the re-aeration rates of seawater as a function of temperature (10- 35° C) applicable to the system and conditions used in this study.

Parameter	$OT (mg l^{-1})$	OTR (mg $l^{-1} h^{-1}$)
k	0.0014 <i>T</i>	0.0009T + 0.0098
Тор	$8.2377 \times \exp(-0.0371T) + 3.6963$	0.0036 <i>T</i>
Bottom	$4.9389 \times \exp(-0.0369T) + 2.2106$	0



FIGURE 1

Background BOD (mg $l^{-1} h^{-1}$) in mulloway (M; triangle) and yellowtail kingfish (KF; circle) tanks adjusted for OTR (mean±se; n=3).



FIGURE 2

Linear relationship between temperature and the mass-specific RMR of mulloway (triangle) and yellowtail kingfish (circle). Data points and solid regression lines represent corrected data (mean \pm se; n = 3). Dashed regression lines representing uncorrected data also shown for comparison.



FIGURE 3

Arrhenius plot of for mulloway (triangle) and yellowtail kingfish (circle) where K = absolute temperature.



FIGURE 4

Relationship between mean Q_{10} (n = 3) and geometric mean temperature for mulloway (triangle) and yellowtail kingfish (circle).

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ABSTRACT

Specific dynamic action (SDA) is the energy expended on the physiological processes associated with meal digestion and is strongly influenced by the characteristics of the meal and the body weight (BW) and temperature of the organism. This study assessed the effects of temperature and body weight on the routine metabolic rate (RMR) and postprandial metabolic response in mulloway, Argyrosomus japonicus, RMR and SDA were established at 3 temperatures (14, 20 and 26°C). 5 size classes of mulloway ranging from 60 g to 1.14 kg were used to establish RMR with 3 of the 5 size classes (60, 120 and 240 g) used to establish SDA. The effect of body size on the mass-specific RMR (mg O_2 kg⁻¹ h⁻¹) varied significantly depending on the temperature; there was a greater relative increase in the mass-specific RMR for smaller mulloway with increasing temperature. No statistical differences were found between the mass exponent (b) values at each temperature when tested against H_0 : b = 0.8. The gross RMR of mulloway (mg O₂ fish⁻¹ h⁻¹) can be described as function of temperature (*T*; 14-26°C) as: $(0.0195T - 0.0454)BW(g)^{0.8}$ and the massspecific RMR (mg O_2 kg⁻¹ h⁻¹) can be described as: $(21.042T - 74.867)BW(g)^{-0.2}$. Both SDA duration and time to peak SDA were influenced by temperature and body weight; SDA duration occurred within 41-89 h and peak time occurred within 17 - 38 h of feeding. The effect of body size on peak metabolic rate varied significantly depending on temperature, generally increasing with temperature and decreasing with increasing body size. Peak gross oxygen consumption (MO_2 : mg O_2 fish⁻¹ h⁻¹) scaled allometrically with BW. Temperature, but not body size, significantly affected SDA scope, although the difference was numerically small. There was a trend for MO_2 above RMR over the SDA period to increase with temperature; however, this was not statistically significant. The average proportion of energy expended over the SDA period (SDA coefficient) ranged from approximately 7 -13% of the total DE intake while the proportion of total energy expended on SDA above RMR ranged from approximately 16 to 27%.

1. INTRODUCTION

The obligatory increase in oxygen consumption (MO_2) that occurs in animals after feeding represents the energy expended on ingestion, digestion, absorption and assimilation of a meal and is often termed specific dynamic action (SDA) (Jobling, 1981; Withers, 1992). In ectotherms, an increase in temperature is generally accompanied by an increase in routine and peak metabolic rates and a decrease in the SDA duration (Robertson et al., 2002; Wang et al., 2003; Luo & Xie, 2008). Body size also influences the SDA response and, in absolute terms, an increase in body size will generally correspond to an increase in metabolic rate, SDA duration and peak metabolism (Tandler & Beamish, 1981; Boyce & Clarke, 1997). The increased O₂ demand associated with feeding has practical implications for the management of intensively cultured aquatic animals. While dissolved O₂ may be at normoxic levels for fish during routine activity, the increased O₂ demand associated with feeding may, depending on stocking densities, induce periods of oxygen debt. If hypoxic conditions occur, voluntary feed intake is reduced (Glencross, 2009) and production potential is then likely to be compromised. The mechanical costs of processing food are considered to be negligible and are in the order of 1 - 3% of the energy expended on SDA (Cho & Slinger, 1979; Peck, 1998) while in most animals, as much as 60-80% of SDA results from post-absorptive metabolism associated with the anabolic cost of protein and lipid synthesis, protein turnover and growth (Wieser, 1994; Willmer et al., 2000). The magnitude and duration of the SDA response is greatly influenced by the characteristics of the meal such as composition (Ross et al., 1992; Peres & Oliva-Teles, 2001; Fu et al., 2007), ration size (Secor & Diamond, 1997; Fu et al., 2005) and feeding frequency (Guinea & Fernandez, 1997; de la Gandara et al., 2002). The proportion of the energy expended during the SDA period above routine metabolism can also vary depending on species (Fu et al., 2005; Fu et al., 2006).

The partitioning and quantification of dietary energy is important in the study of nutritional energetics because it provides a convenient platform to predict the energy balance of individuals based on body weight, sex, activity, physiological state, environment, and amount and nutritive value of feed eaten (Baldwin & Bywater, 1984). This information can then form the basis for diet formulation and evaluation (Bureau et al., 2002). Energy exchanges in biological systems can be studied in terms of their biochemical thermodynamics or bioenergetics, which involves the examination of energy gains, losses and transfers within the whole organism (Jobling, 1994; Haynie, 2001). Energy budgets account for the energy ingested (IE) and the energy used for metabolism (M), nitrogenous waste (UE), fecal waste (FE) and production (P; somatic and non-somatic growth). This can be expressed in the general form:

IE = M + UE + FE + P

M can be further partitioned as the sum of routine metabolic rate (RMR; basal metabolism + metabolism associated with routine activity) + SDA.

The energetic requirements for RMR, maintenance and growth have been established for mulloway (Pirozzi & Booth, 2009; Pirozzi et al., 2010); however, information on the allometric relationships with temperature and metabolism are limited and there is currently no information on the SDA response for this species. The objectives of this study were to describe the influence of body mass and temperature on the RMR and SDA of mulloway.

2. MATERIALS AND METHODS

2.1 Experiment design

The influence of temperature on oxygen consumption (MO₂) was tested using five size classes of juvenile mulloway (mean initial body weight (g) \pm SD; 60.4 \pm 0.9, 122.2 \pm 2.6, 240.5 \pm 3.6, 496.7 \pm 2.5 or 1140.6 \pm 1.6). Sizes classes are referred to as XS, S, M, L or XL respectively. Fish were stocked in triplicate groups for each of the 3 temperature treatments (14, 20 or 26°C) into 200 l open-top tanks at n = 22, 12, 8, 3 or 2 fish per tank for the XS, S, M, L and XL fish respectively. Stocking densities were chosen to mitigate the potential for density dependent behavioral effects (Pirozzi et al., 2009).

All fish (i.e. all temperature x size groups) were initially stocked into the experiment system at ambient water temperature (16°C) and then adjusted 1°C day⁻¹ until the start temperature of 14°C was reached. Fish were then held for two weeks at that temperature to acclimate to the system before MO_2 readings were taken for the 14 °C treatment group. During acclimation all fish were fed a maintenance ration once daily of a 6mm sinking commercial diet (Ridley AquaFeed Pty. Ltd., Narangba, Qld. Australia; 45.5% crude protein, 18.7% crude fat, 22.2 MJ kg⁻¹ gross energy). The apparent digestibility coefficient for energy of the diet was 0.84 (Booth, unpublished data, 2008). Each tank was supplied with constant water flow (6 1 min⁻¹) and air when MO_2 was not being recorded. Tanks were exposed to

indirect natural lighting (photoperiod 11L:13D) and water quality parameters (pH 7.5-7.84; $NH_4^+ < 0.1 \text{ mg/L}$; salinity 30.6-33.0 ppt) were monitored regularly.

2.2 MO₂

 MO_2 readings were established as per Pirozzi & Booth (2009). Fish were fasted for 96, 72 or 48 h depending on the temperature treatment (i.e. 14, 20 or 26 °C respectively) prior to establishing routine metabolic rates (RMR). MO_2 measurements for each temperature were repeated three times over approximately 2 h intervals for each replicate tank and the mean regarded as the RMR. After RMR was established fish were fed the commercial diet slowly from 16:30 over approximately 1 h to slightly in excess of apparent satiation. Any uneaten pellets remaining after the feeding period were counted then siphoned from tanks. Total feed intake was then adjusted accordingly using a predetermined individual pellet weight of 0.21 ± 0.02 g (mean \pm SD; n = 202). MO_2 during the SDA period was monitored up to 72 h or until MO_2 rates fell within the standard error of RMR levels. MO_2 rates remained elevated after 72 h for the mulloway at 14°C however readings taken at day 6 post-feeding showed that MO_2 had returned to RMR levels. The L and XL size mulloway did not feed well and were excluded from SDA analyses.

Fish that were assigned as the 14°C treatment were re-weighed after MO_2 readings were completed for that temperature and removed from the system. Background biochemical oxygen demand (BOD) was determined for each replicate tank after fish were removed and water had been re-saturated with O_2 . The temperature was then adjusted up 1°C day⁻¹ until the next experiment temperature (20°C) was reached. The remaining fish in the system were acclimated for a further week before MO_2 was recorded. This protocol was again repeated for the final temperature (26°C). After MO_2 measurements had been completed a subsample of 5 individual fish from each replicate tank were euthanized with an overdose of benzocaine (ethyl-p-aminobenzoate) and dissected to determine the presence or absence of feed remaining in the digestive tract. Fish from the 14°C treatment were sub-sampled on day 6 post-feeding.

2.3 RMR and SDA Parameters

Mass-specific MO₂ was calculated as:

$$(V/BW/n) \times (\varDelta O_2 - O_{2otr} + O_{2bod})$$

Where V = tank water volume (l); BW = mean body weight (kg); n = number of fish tank⁻¹; ΔO_2 = net change in O₂ concentration (mg l⁻¹ h⁻¹) inclusive of fish respiration, atmospheric re-aeration and background BOD; O_{2otr} = atmospheric oxygen transfer rate (OTR; mg l⁻¹ h⁻¹); O_{2bod} = background BOD rate (mg l⁻¹ h⁻¹). OTR was calculated using seawateratmosphere O₂ transfer coefficients established in a separate study (Pirozzi & Booth, 2009).

The following MO₂ and SDA indices were calculated:

 MO_{2rmr-g} : Routine metabolic rate (RMR) expressed as gross MO_2 (mg O_2 fish⁻¹ h⁻¹) defined as the metabolic rate associated with standard metabolism and spontaneous swimming activity of post-absorptive fish fasted for 48 h – 96 h (depending on temperature treatment)

 MO_{2rmr-s} : Mass-specific RMR (mg O_2 kg⁻¹ h⁻¹)

 MO_{2sda-d} : SDA duration (h) defined as the time from initial feeding to the point when MO_2 rates returned to within the SE of MO_{2rmr-s} . 14°C treatments were estimated by fitting a quadratic function and deriving the *x*-intercept when y = +SE of MO_{2rmr-s}

- MO_{2sda-p}: Peak post-prandial MO₂ derived as *y*-intercept of asymptote of quadratic function fitted to MO_{2sda-d}
- $MO_{2sda-pd}$: SDA duration from feeding to peak post-prandial MO_2 derived as x-intercept of asymptote of quadratic function fitted to MO_{2sda-d}
- MO_{2scope} : Factorial scope calculated as $MO_{2sda-p} MO_{2rmr-s}^{-1}$
- MO_{2sda}: The cumulative measurement of MO₂ (mg O₂ kg⁻¹) above RMR over the SDA period. Calculated as the area under the curve (AUC) (Motulsky and Christopoulos, 2003). 14°C treatment was estimated based on predicted MO_{2sda-d}.
- SDA coefficient: Estimate of the proportion (%) of total digestible energy (DE) intake from feed expended on SDA. Based on an oxyenergetic conversion factor of 13.59 kJ mg O_2^{-1} (Elliott and Davison, 1975) applied to MO_{2sda} .
- SDA_E: Proportion (%) of SDA energy expenditure above RMR
- TE: Total metabolic expenditure (kJ kg^{-0.8}) over SDA period (sum of SDA + RMR)

2.4 Data analyses

The effect of temperature on the MO_{2rmr-s} and MO_{2rmr-g} of the 5 different size classes of mulloway (XS, S, M, L, XL) was tested with 2-way ANOVA (temperature x size). SDA variables were compared between 3 sizes (XS, S, M) using 2-way ANCOVA (temperature x size) with relative feed intake (RFI: g feed BW kg⁻¹) as the co-variate. Two-way ANOVA was used on SDA variables where the covariate was not significant (MO_{2sda-d} , MO_{2scope}). RFI was also compared between XS, S and M size mulloway using 2-way ANOVA. All data were normally distributed according to skewness, kurtosis and omnibus normality tests (NCSS 2004, Kaysville, Utah). All variances were homogeneous according to modified Levenes' equal variance test. Tukey-Kramer test was used for *a posteriori* multiple comparison of means on significant terms. Comparisons of individual model parameters were made using the extra sum-of-squares *F*-test. Results of all statistical tests were regarded as significant at p<0.05.

The effect of body weight on RMR at each temperature was described by the allometric equation:

 $RMR = aBW^b$

Where a is the normalizing constant, BW is the body mass in g and b is the scaling exponent describing the influence of mass on metabolism. Power functions were iteratively derived using the non-linear least squares method in Graphpad Prism[®] v 4.0.

The SDA responses for XS, S and M size mulloway at each temperature were fitted with a quadratic function in the form:

 $MO_{2SDA} = a + bt + ct^2$

Where MO_{2SDA} is the mass-specific MO_2 (mg O_2 kg⁻¹ h⁻¹) over the SDA period expressed as function of time (t) in hours.

3. **RESULTS**

3.1 Temperature and fish size interactions

3.1.1 RMR

The effect of body size on the MO_{2rmr-s} of mulloway varied significantly depending on the temperature (Table 1). There was a significantly greater relative increase in MO_{2rmr-s} for smaller mulloway with increasing temperature (Table 2). Partitioning of the data demonstrated no significant interaction (ANOVA; temperature x size; p>0.5) between MO_{2rmr-s} at 20 and 26°C while comparisons at 14 and 20°C were significant (temperature x size; p<0.01). The interaction with size and temperature is reflected in the different scaling exponent (*b*) values (Table 3, Figure 1). Exponent values for MO_{2rmr-g} at 20 and 26°C were very similar and were higher at 14°C (Table 3). However; no significant difference was found between each exponent value when tested against H_0 : b = 0.8 (p>0.2, 0.05 and 0.1 for 14, 20 and 26°C respectively). The gross RMR of mulloway (mg O_2 fish⁻¹ h⁻¹) can therefore be described as function of temperature (*T*; 14-26°C) as:

$$MO_{2rmr-g} = (0.0195T - 0.0454)BW(g)^{0.8}$$
 (r² = 0.999) 1

and the mass-specific RMR (mg O_2 kg⁻¹ h⁻¹) can be described as:

$$MO_{2rmr-s} = (21.042T - 74.867)BW(g)^{-0.2}$$
 (r² = 0.99) 2.

3.1.2 SDA

Figure 2 shows the SDA responses for XS, S and M size mulloway at 14, 20 and 26°C. RFI was a significant covariate for MO_{2sda-p} , MO_{2scope} and MO_{2sda} (Table 4). No food was present in the digestive tract of any size mulloway at any temperature at the conclusion of the study.

The effect of body size on MO_{2sda-p} varied significantly depending on temperature (Table 4). MO_{2sda-p} generally increased with temperature and decreased with increasing size (Table 5). The relationship between peak gross MO_2 (mg O_2 fish⁻¹ h⁻¹) and BW was allometric (Figure 3) and can be described at each temperature as:

14° C (mg O ₂ fish ⁻¹ h ⁻¹) = 0.144BW(g) ^{0.938}	$(r^2 = 0.989)$	3.
20° C (mg O ₂ fish ⁻¹ h ⁻¹) = 0.321BW(g) ^{0.882}	$(r^2 = 0.998)$	4.
26° C (mg O ₂ fish ⁻¹ h ⁻¹) = 0.692BW(g) ^{0.799}	$(r^2 = 0.985)$	5.

Exponent values for peak gross MO_2 at 14 and 20°C differed significantly (P<0.0001) from 0.8.

Temperature and body size significantly, but independently, influenced $MO_{2sda-pd}$ and MO_{2sda-d} (Table 6). MO_{2sda-d} ranged from approximately 41 – 89 h and generally decreased with increasing temperature and increased with increasing size (Table 5). $MO_{2sda-pd}$ ranged from 17 – 38 h. The relationship between temperature and both $MO_{2sda-pd}$ and $MO_{2sda-pd}$ was linear (Figure 4) with duration decreasing with increasing temperature. There was no effect of temperature between slopes (p>0.5) with regard to MO_{2sda-d} however the y-intercepts differed significantly (p<0.05). Therefore, when slope = -3.467 ($r^2 = 0.93$), $MO_{2sda-d} = 128.4$ ($r^2 = 0.96$), 132.7 ($r^2 = 0.92$) and 135.3 h ($r^2 = 0.91$) at x = 0 for XS, S and M size mulloway respectively.

The relationship between temperature (*T*; 14-26°C) and $MO_{2sda-pd}$ for XS, S and M size mulloway can be described as:

XS = -1.185T + 48.36	$(r^2 = 0.94)$	6.
S = -0.914T + 42.44	$(r^2 = 0.87)$	7.
M = -1.470T + 58.07	$(r^2 = 0.94)$	8.

Temperature, but not body size, significantly affected MO_{2scope} (Table 4). MO_{2scope} demonstrated a small but statistically significant increase of, on average, 0.16 at 26°C ($MO_{2scope} = 1.51$) compared to both 14 and 20°C ($MO_{2scope} = 1.37$, 1.34 respectively) (Table 6).

There was a trend for MO_{2sda} to increase with temperature (Table 5); however, this was not significant (Table 4). MO_{2sda} was, on average, 1812, 1967 and 2799 mg at 14, 20 and 26°C respectively (Table 5). There was no obvious trend indicating the relationship between MO_{2sda} and size (Table 5).

The influence of temperature on RFI and the SDA coefficient varied significantly depending on the body size of mulloway (Table 6). The average proportion of energy expended on SDA ranged from approximately 7 - 13% of the total DE intake (Table 5).

Temperature, but not body size, significantly influenced SDA_E (Table 6) with the greatest SDA energy expenditure above RMR occurring at 26°C.

When expressed independent of mass, total energy expenditure (TE) (kJ kg^{-0.8}) increased linearly with relative energy intake (kJ DE kg^{-0.8}) (Figure 5). Post-hoc comparisons between regressions of each temperature treatment indicated that one set of global parameters could be used to describe the data for 20 and 26°C (p>0.1). The slopes of the regression did not differ significantly among the three temperature treatments (p>0.5), consequently a common regression coefficient can be used across all temperatures:

TE (20-26°C; kJ kg ^{-0.8}) = $0.068DE + 72.88$	$(r^2=0.55; n=18) 9.$
TE $(14^{\circ}\text{C}; \text{kJ kg}^{-0.8}) = 0.068\text{DE} + 64.00$	(<i>r</i> ² =0.45; n=9) 10.

3.2 MO_{2SDA} Curve fitting

Quadratic equations over the MO_{2sda-d} responses (Figure 2) are given below. Estimates for the 14°C treatment have poor coefficient of determination (r^2) values as estimates were based on data collected up to 72 h post feeding and before the full SDA response was completed.

XS mulloway		
$MO_{2SDA} (14^{\circ}C) = 105.9 + 0.75t - 0.012t^{2}$	$(r^2 = 0.26)$	11.
$MO_{2SDA} (20^{\circ}C) = 176.1 + 2.11t - 0.041t^{2}$	$(r^2 = 0.57)$	12.
$MO_{2SDA} (26^{\circ}C) = 241.5 + 8.12t - 0.227t^{2}$	$(r^2 = 0.68)$	13.
S mulloway		
$MO_{2SDA} (14^{\circ}C) = 96.0 + 0.54t - 0.009t^{2}$	$(r^2 = 0.18)$	14.
$MO_{2SDA} (20^{\circ}C) = 158.5 + 1.72t - 0.0341t^{2}$	$(r^2 = 0.59)$	15.
$MO_{2SDA} (26^{\circ}C) = 214.0 + 4.75t - 0.129t^{2}$	$(r^2 = 0.70)$	16.
M mulloway		
$MO_{2SDA} (14^{\circ}C) = 84.9 + 0.96t - 0.013t^{2}$	$(r^2 = 0.29)$	17.
$MO_{2SDA} (20^{\circ}C) = 139.0 + 2.12t - 0.037t^{2}$	$(r^2 = 0.58)$	18.
$MO_{2SDA} (26^{\circ}C) = 177.2 + 5.12t - 0.127t^{2}$	$(r^2 = 0.61)$	19.

4.1 Influence of mass and temperature on RMR

In fish, the mass scaling exponent of resting metabolic rate has been shown to be approximately 0.8 (Winberg, 1956; Clarke & Johnston, 1999) although *b* is known to vary with temperature in some species (Beamish, 1964; du Preez et al., 1986; Xie & Sun, 1990; Hölker, 2003). The influence of body size on the RMR of mulloway was also shown to vary depending on temperature which was reflected in *b* which varied from 0.73-0.85. However, as *b* did not vary significantly from 0.8, this value can therefore be considered as appropriate to describe the influence of body mass on the routine metabolism of mulloway over the temperature range used in this study. By constraining *b* important biological variability may be obscured (Clarke & Johnston, 1999), however, *a* then exclusively describes the influence of temperature, and experimental conditions, on metabolic rate and the error associated with *a* is considerably reduced (Table 3). Equations 1 and 2 provide predictions of the routine metabolic demand for O₂ by juvenile mulloway based on BW and temperature (14 to 26° C) and give estimations which are very close to those published for this species using different size fish than those used in the current study (Fitzgibbon et al., 2007b; Pirozzi & Booth, 2009).

It is important to consider where in the metabolic scope of activity measurements are derived (i.e. from basal through to maximal metabolic rate). In mammals b is positively correlated with increasing levels of metabolic activity (Savage et al., 2004; Weibel et al., 2004; White & Seymour, 2005a) and similar trends were observed with mulloway in the current study; across all temperatures b increased with the increased metabolic activity of peak MO_2 associated with SDA. In mulloway the relationship between mass and metabolism appears to become less allometric and more isometric (i.e. as b approaches 1) with increasing levels of metabolic activity, particularly at lower temperatures. However, this relationship requires further validation as the size range of mulloway used in the present study may influence the value of b. The conditions in which data are derived and the size ranges of animals used are known to effect exponent values (White and Seymour, 2005b) emphasizing the importance of standardizing experimental procedures, clearly defining the metabolic level being measured and, consequently, raising caution when attempting to make inter or intraspecific comparisons across different studies.

4.2 SDA response

4.2.1 SDA duration

The SDA responses in mulloway were typical of those exhibited by other fish and ectothermic species: metabolic rate increased following ingestion of feed and then gradually declined over a number of days with the duration of the SDA and peak time response markedly affected by both temperature and body size (Jobling & Davies, 1980; Boyce & Clarke, 1997; McCue, 2006; Secor, 2009). SDA durations of approximately 40 to 90 h for mulloway fall within the ranges reported for other temperate fish species (reviewed by McCue, 2006) but are much lower than some Antarctic species which are reported at 240-390 h at ~0°C (Boyce & Clarke, 1997). Gastric evacuation time is strongly correlated with SDA duration (Jobling & Davies, 1980) and this was indicated in mulloway at each temperature with the absence of feed in the digestive tract of fish sub-sampled at the conclusion of MO_2 readings. Although MO_2 readings at 14°C were ended at 72 h, and before the full SDA duration was completed, the absence of feed combined with MO_2 rates which had returned to RMR levels at day 6 post-feeding lends support to our estimation of approximately 80-90 h SDA duration at this temperature.

4.2.2 SDA factorial scope

While body size clearly influences the overall SDA duration, it has been shown to have little effect on the post-prandial factorial scope in fish (Jobling & Davies, 1980; Johnston & Battram, 1993) which was also confirmed with mulloway in this study. Temperature is also considered not to have a large influence on factorial scope (Jobling & Davies, 1980; Johnston & Battram, 1993) however mulloway were shown to have a significantly greater factorial scope at 26°C than at 14 or 20°C. The largest difference between temperature treatments however, although statistically significant, was on average 0.17. This tends to support the conclusions of other studies that the influence of temperature on factorial scope is quite small. It should be noted however, that factorial scope is a relative unit expressed as a multiple of RMR levels and large differences can be seen in peak MO_2 between treatments when expressed in absolute terms (Peck, 1998; Figure 2). The factorial scope of mulloway demonstrated in the current study (1.3-1.5) is at the low end compared to those for other fish species which can range from 1.4 - 4.1 (see review by McCue, 2006) however; there are several contributing reasons for this. Firstly, the magnitude of the SDA response is greatly affected by meal size (Hamada & Maeda, 1983; Boyce & Clarke, 1997; Fu et al., 2005). In this study mulloway ate approximately 2% of their body weight which, although typical for this species fed to satiation on the type of feed used in this study (Pirozzi et al., 2010), may be considered small compared to other species such as southern catfish (Silurus meridionalis) which have a correspondingly higher factorial scope of 4.1 when the relative meal size is 24% (Fu et al., 2005). Secondly, the factorial scope in the current study is reported relative to RMR which will vary among species depending on their normal resting or routine level of activity. Thirdly, factorial scope is sometimes reported relative to standard or basal metabolic rates (e.g. Beamish, 1974; Chen et al., 2008) which will increase values. Lastly, our values for mulloway are derived from the models fitted to the data and will therefore slightly underestimate the maximal recorded values.

4.2.3 SDA coefficient and energy expenditure

At approximately 7-13%, the average SDA coefficient for mulloway, i.e. the energy devoted to the SDA response as proportion of the energetic content of a meal, was within the range reported for most temperate fish species (6-23%) (Pandian & Vivekanandan, 1985; McCue, 2006). The SDA coefficient is known to be influenced by body size (Beamish, 1974), meal size (Carter & Brafield, 1992; Fu et al., 2006), meal type (Secor & Boehm, 2006) and, in the case of mulloway, the influence of body size on the SDA coefficient varied depending on temperature which was likely due to the corresponding interaction between body size and temperature on relative feed intake (Table 6). The influence of these variables on the SDA coefficient therefore makes direct comparison amongst other studies very difficult (Beaupre, 2005; McCue, 2006). Expressing total energy expenditure as a function of DE intake independent of body weight (Figure 5) perhaps gives a somewhat better insight into SDA energetics as it at least avoids the potential confounding caused by the allometric relationships associated with body mass and meal size inherent when making comparisons of coefficients derived from mass-specific data (Beaupre, 2005). When expressed this way total energy expenditure by mulloway was shown to increase linearly with increasing DE intake. Temperature is generally considered to have little influence on SDA expenditure (see reviews by McCue, 2006; Secor, 2009) although temperature effects have been noted in some fish species (Guinea & Fernandez, 1997; Peck et al., 2003; Luo & Xie, 2008). Although values for MO_{2sda-p}, MO_{2sda-pd} and MO_{2sda-d} all differed among temperatures irrespective of the size class of mulloway, energy expenditure (kJ kg^{-0.8}) relative to intake (kJ kg^{-0.8}) was shown to be very similar at 20 and 26°C and approximately only 9 kJ kg^{-0.8} less at 14°C. The absolute difference among these temperatures remained constant because the DE utilization efficiency was approximately the same at all temperatures for any given quantity of feed (see also Pirozzi et al., 2010). The difference in magnitude however will decrease exponentially with increasing feed intake from approximately 12% difference at zero intake to 8% difference at an intake of $500 \text{ kJ kg}^{-0.8}$.

Mulloway are a eurythermal species typically found in warm-temperate to sub-tropical estuaries and near-shore waters (Harrison & Whitfield, 2006; Silberschneider & Gray, 2008) where temperatures of 20 or 26°C are not uncommon (Harrison, 2004; Harrison & Whitfield, 2006). If metabolic rates are dependent on the temperature-sensitive properties of enzymes and cellular components which in turn determine thermal optima (Hochachka & Somero, 2002), the similar net response on energy expended due to SDA by mulloway at 20 and 26°C may be indicative of the biochemical rate processes operating within a thermal range suitable for normal metabolic function. In ectotherms there is a negative correlation between peak SDA metabolism and the duration of the SDA response which is dependent on temperature (McCue, 2006). When peak SDA increases there is a corresponding decrease in SDA duration; the resultant net energy expenditure being similar (Wang et al., 2003; Secor et al., 2007; Luo & Xie, 2008). This response was seen with mulloway and is typical of most temperature performance curves recorded for ectotherms (Angilletta et al., 2002) and demonstrates the trade off between the "specialist" (high narrow peak) and the "generalist" (low broad peak) metabolic responses (see Huey & Hertz, 1984; Gilchrist, 1995).

It is important to note that RMR represents the major proportion of the total energy expended during the SDA period (Tables 6), the greatest proportion of SDA above RMR occurred at 26°C accounting for approximately 27% of the total energy expenditure and is likely to be related to the energetic cost incurred for increased protein turnover and synthesis (Houlihan et al., 1988; Brown & Cameron, 1991). This indicates a greater potential for growth at this temperature which has been confirmed in other feeding studies with mulloway (Pirozzi et al., 2010). Although mulloway are a relatively sedentary species, the above values demonstrate a relatively high proportion of DE intake dedicated to maintaining routine metabolism and, although comparable to values reported for some teleost species (Carter & Brafield, 1992; Xie et al., 1997; Owen, 2001), indicates a moderate scope for growth particularly when compared to the high-energy-demand species such as the yellowtail (*Seriola quinqueradiata*) (Watanabe et al., 2000) and southern bluefin tuna (*Thunnus maccoyii*) (Fitzgibbon et al., 2007a).

4.2.4 Conclusion

RMR and SDA were shown to represent significant energetic costs in the overall energy budget of mulloway. Many of the SDA indices measured in this study were within the ranges of those reported for other temperate marine fish; however, we have demonstrated that these values are not fixed and are highly dependent on temperature, body size and feed intake. We have therefore presented equations as a function of these variables which will allow greater accuracy in the bioenergetic modeling of metabolic expenditure for this species.

If the greatest proportion of SDA energy is channeled towards the biochemical processes that contribute to growth (Wieser, 1994; Willmer et al., 2000), it would then appear that the growth rate potential of mulloway may be limited at 14 and 20°C (compared to 26°C). The gathering body of information on the temperature responses of various metabolic, growth and preference parameters measured for mulloway thus far indicate that a temperature of approximately 26±2°C to be optimal for growth and metabolic function (Bernatzeder and Britz, 2007; Collett et al., 2008; Pirozzi & Booth, 2009; Pirozzi et al., 2010). It is not known what the SDA response in mulloway is at temperatures above 26°C; however, there are indications the SDA coefficient may be reduced in some ectotherms at elevated temperatures (Cui & Wootton, 1988; Toledo et al., 2003).

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2-way ANOVA on MO_{2rmr-s} and MO_{2rmr-g} for all sizes classes. ns = not significant at p<0.05, *=p<0.05, **=p<0.01, ***=p<0.001

Source of		MO _{2rmr-s}			MO _{2rmr}	-g	
Variation	DF	MS	F	Р	MS	F	Р
A:							
Temperature	2	28290.2	377.4	***	2159.5	118.1	***
B: Size	4	5755.3	76.78	***	8648.6	473.1	***
AB	8	359.1	4.8	***	209.6	11.5	***
Residual	30	75.0			18.3		

Summary of RMR MO_2 results (mean±se) for XS – XL size mulloway at 14, 20 and 26°C. ANOVA on final BW data analyzed within size class and were significant at p<0.05 but not p<0.01. Means sharing superscript letters are not significantly different (p>0.05) according to Tukey-Kramer test.

	XS		S	S			M L			L			XL		
Variable	14°C	20°C	26°C	14ºC	20°C	26°C	14°C	20°C	26°C	14°C	20°C	26°C	14ºC	20°C	26°C
BW (g)	61.6±0.3	60.4±2.1	60.9±0.6	123.5±2.4	119.2±3.3	120.9±2.0	248.8±3.0 ^a	235.5±1.4 ^b	245.7±3.6 ^{ab}	478.6±6.4ª	444.4±5.0 ^b	439.2±9.5 ^b	1134±45 ^a	1011±18 ^b	976±3 ^b
MO _{2rmr-g} (mg O ₂ fish ⁻¹ h ⁻¹)	5.6±0.1ª	9.7±0.2 ^{ab}	12.4±0.2 ^{ab}	9.4±0.1 ^{ab}	16.4±0.2 ^{abc}	21.2±0.5 ^{bcd}	17.7±0.5 ^{abc}	28.2±2.0 ^{cde}	37.1±1.3 ^{ef}	31.4±3.0 ^{de}	49.7±2.3 ^f	67.1±3.4 ^g	63.9±5.2 ^g	83.3±4.8 ^h	110.1±3.0 ⁱ
MO _{2rmr-s} (mg O ₂ kg ⁻¹ h ⁻¹)	90.8±1.9 ^{bc}	160.8±7.9 ^{ef}	203.2±4.5 ^g	75.8±2.0 ^{ab}	138.1±3.2 ^{de}	175.4±2.2 ^f	71.1±2.7 ^{ab}	119.9±9.0 ^{cd}	151.0±3.6 ^{ef}	65.6±5.7 ^{ab}	111.9±5.8°	152.6±5.8 ^{ef}	56.2±2.7ª	82.3±3.4 ^b	112.8±2.9 ^{cd}

Parameters of the power function $y = aM^b$ describing the relationship between body mass and MO_{2rmr-g} or MO_{2rmr-s} for mulloway at each experiment temperature. Data shown for iteratively derived parameters and also for coefficient values when b fixed at 0.8 (MO_{2rmr-g}) or -0.2 (MO_{2rmr-s}).

RMR	Temperature	Unconstrained b			Constrained b	
variable	(°C)	a±se	<i>b</i> ±se	r^2	a±se	r ²
MO _{2rmr-g}	14	0.158±0.05	$0.854{\pm}0.04$	0.98	0.228±0.01	0.98
	20	0.536±0.12	0.732 ± 0.04	0.98	0.341±0.01	0.98
	26	0.622±0.13	0.754±0.03	0.99	0.461 ± 0.01	0.99
MO _{2rmr-s}	14	174.2±22.4	-0.161±0.02	0.78	212.5±5.3	0.74
	20	394.4±45.7	-0.218 ± 0.02	0.89	360.4±7.3	0.88
	26	440.6±45.6	-0.190±0.02	0.90	465±7.4	0.90

2-way ANCOVA on MO₂ SDA variables for XS, S and M size mulloway with RFI as a significant co-variate. ns = not significant at p<0.05, * = p<0.05, ** = p<0.01, *** = p<0.001

Source of		MO _{2sda-p}			MO _{2scope}			MO _{2sda}		
Variation	DF	MS	F	P	MS	F	P	MS	F	Р
X(RFI)	1	384.3	12.3	**	0.035	4.8	*	884959	5.6	*
A: Temperature	2	7703.9	246.6	***	0.046	6.3	**	562757	3.5	ns
B: Size	2	4048.0	129.6	***	0.004	0.5	ns	247352	1.6	ns
AB	4	547.7	17.5	***	0.007	0.9	ns	362705	2.28	ns
Residual	17	31.2			0.007			159119		

Summary of SDA MO₂ results (mean±se) for XS, S and M size mulloway at 14, 20 and 26°C. All data analyzed by ANOVA except for MO_{2sda-p} , MO_{2scope} and MO_{2sda} which were analyzed using ANCOVA (RFI as covariate). Means sharing superscript letters are not significantly different (p>0.05) according to Tukey-Kramer test.

	XS			S			Μ		
Variable	14°C	20°C	26°C	14°C	20°C	26°C	14°C	20°C	26°C
RFI (g kg ⁻¹)	10.8±0.4 ^a	19.9±1.8 ^{bc}	24.5±0.4 ^c	11.1±0.6 ^a	16.8±1.3 ^{ab}	23.3±0.4 ^{bc}	21.3±1.2 ^{bc}	24.8±2.0 ^c	26.2±2.0 ^c
MO _{2sda-p} (mg O ₂ kg ⁻¹)	117.7±2.6 ^b	203.2±3.8 ^e	313.5±3.0 ^g	103.9±1.7 ^b	180.5±3.3 ^d	257.6 ± 4.0^{f}	102.7±3.5 ^a	169.2±1.6 ^c	229.0±8.8 ^e
MO _{2sda-d} (h)	78.8±3.0 ^{de}	57.9±1.3 ^{bc}	40.5±1.5 ^a	86.2±5.6 ^e	60.5±0.9 ^c	43.6±0.3 ^{ab}	88.9±5.9 ^e	64.1±2.4 ^{cd}	44.9±1.3 ^{ab}
MO _{2sda-pd} (h)	31.2±1.1 ^d	25.7±0.9 ^{cd}	17.0±0.4 ^a	29.3±0.7 ^{cd}	24.8±1.9 ^{bc}	18.4±0.1ª	37.7±1.8 ^e	28.3±1.1 ^{cd}	$20.1{\pm}1.0^{ab}$
MO _{2scope} (MO _{2sda-p} MO _{2rmr-s} ⁻¹)	1.30±0.02 ^{ab}	1.27±0.02 ^a	1.54±0.03 ^{ab}	1.37±0.03 ^b	1.31±0.03 ^{ab}	1.47±0.03 ^{ab}	1.45±0.06 ^{ab}	1.43±0.13 ^{ab}	1.52±0.04 ^{ab}
MO _{2sda} (mg O ₂ kg ⁻¹)	1586±139 ^{ab}	1756±227 ^a	3371±200 ^b	1827±65 ^{ab}	1844±148 ^{ab}	2555±173 ^{ab}	2022±154 ^a	2299±587 ^a	2472±252 ^a
$\mathrm{SDA}_E(\%)$	18.1±1.0 ^{ab}	15.9±2.2 ^a	29.0±0.8 ^b	22.0±1.5 ^{ab}	18.1±1.3 ^{ab}	25.0±1.4 ^{ab}	24.5±2.8 ^{ab}	22.8±5.2 ^{ab}	26.6±1.4 ^{ab}
SDA co-ef (%)	11.5±0.9 ^{abc}	7.1±1.4 ^a	10.7±0.5 ^{abc}	12.8±0.6 ^c	8.6±0.8 ^{ab}	8.5±0.5 ^{ab}	7.4±0.2 ^{ab}	7.0±1.3 ^a	7.3±0.4 ^{ab}

2-way ANOVA on SDA variables for XS, S and M size mulloway with RFI as a non-significant covariate. Results of 2-way ANOVA for RFI also shown. ns = not significant at p<0.05, * = p<0.05, ** = p<0.01, *** = p<0.001

Source of	MO _{2sda-pd}			MO _{2sda-d}				SDA coef			\mathbf{SDA}_E			RFI		
Variation	DF	MS	F	Р	MS	F	Р	MS	F	P	MS	F	Р	MS	F	Р
A:																
Temperature	2	459.6	118.5	***	3920.6	134.7	***	19.9	9.4	**	146.9	8.9	***	240.4	47.1	***
B: Size	2	55.0	14.2	***	108.8	3.7	*	20.9	9.9	**	33.0	2.0	ns	126.0	24.7	***
AB Residual	4 18	9.6 3.9	2.5	ns	8.5 29.1	0.3	ns	7.2 2.1	3.4	*	23.5 16.4	1.4	ns	18.0 5.1	3.5	*



FIGURE 1

Relationship between BW (g) and A. MO_{2rmr-g} and B. MO_{2rmr-s} at 14 (squares), 20 (triangles) and 26°C (circles). Solid lines represent power functions with exponent (b) values fixed at 0.8 and -0.2 for graphs A and B respectively. Dashed lines represent power functions with unconstrained iteratively derived b. Refer to Table 3 for specific parameter values.



FIGURE 2

Temporal pattern of SDA measured as mean MO_2 (mg O_2 kg⁻¹ h⁻¹) (±se; n = 3) of XS, S and M size mulloway at 14 (squares), 20 (triangles) and 26°C (circles). Horizontal dashed lines represent MO_{2rmr-s} at each temperature and size treatment. Refer to Table 2 for MO_{2rmr-s} values. Quadratic functions shown fitted for MO_{2sda-d}



FIGURE 3 Allometric relationship between BW (g) and peak gross MO_2 (mg fish⁻¹ h⁻¹) (n = 9).


Linear relationship between temperature and A. $MO_{2sda-pd}$ and B. MO_{2sda-d} (±se; n = 3) for XS (circles), S (triangles) and M (squares) size mulloway.



Total energy expenditure over the duration of the SDA response (kJ kg^{-0.8}) as a function of increasing DE intake (kJ kg^{-0.8}). Regression lines shown for 20 and 26°C (global $r^2=0.55$; n=18; solid line) and 14°C ($r^2=0.45$; n=9; dashed line). Refer to Eqs. 9 and 10 for regression parameter values. Energy expenditure at zero intake estimated from the proportion of cumulative RMR (n=27) over the SDA duration shown for comparison and not used to formulate regression lines.

4.10 A factorial approach to deriving diet formulations and daily feed intake for mulloway, *Argyrosomus japonicus*, based on the requirements for digestible protein and energy.

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ABSTRACT

This study applied a factorial approach to predicting the requirements for digestible protein (DP) and digestible energy (DE) for mulloway throughout the production range. Published data relating to protein and energy utilization and protein and energy requirements for maintenance and growth of this species were consolidated with quantitative descriptions of proximate whole body composition and an assessment of growth potential undertaken over a range of temperature and fish sizes. Factorial modelling of the data provided estimations of the decreasing requirement of the ratio of DP:DE for mulloway with increasing body size up to 2 kg. Piecewise regression analyses identified significant changes in the requirement for DP:DE at key growth stages. From this information diet formulations and suggested daily feed intake were iteratively derived applicable for the different dietary requirements dependant on body size. Four growth stages with corresponding dietary requirement for DP:DE are suggested; 10-100 g = 31.3 g DP MJ DE⁻¹, 100-500 g = 24.8 g DP MJ DE⁻¹, 500-1100 g = 20.8 g DP MJ DE⁻¹, 1100-2000 g = 19.1 g DP MJ DE⁻¹. Sensitivity analyses was used to test the response of the factorial model to small perturbations of individual parameter values on the predicted optimal ratio of DP:DE. Protein and energy utilisation coefficients and the whole body composition coefficients for protein and energy were identified to have the greatest influence on the predicted requirement for DP:DE while the growth model exponent value becomes increasingly influential for fish >200 g.

Keywords: Mulloway; Factorial modeling; Growth; Body composition; Feed evaluation system

1. Introduction

Nutrient requirements in fish have traditionally been determined empirically using a doseresponse approach, typically with weight gain or nutrient retention expressed as the response criteria and the relationship analysed using regression analyses. Evaluating diets by testing all combinations of nutrient inclusion levels against various response criteria and under various culture conditions will undoubtedly yield the most accurate definitions; however, this approach is neither cost effective nor practical to implement. Mathematical modelling in animal nutrition provides an extremely useful tool in the development of practical feed evaluation systems (i.e. feeding standards and practices) to describe and predict nutrient requirements, body composition and growth of the animal (Cho, 1992; Dijkstra et al., 2007). Bioenergetics is the quantitative study of energy gains, losses and transfers within the whole organism based on thermodynamic principles (Bureau et al., 2002; Haynie, 2001; Jobling, 1994), and has been widely applied to animal nutrition and the development of feed evaluation systems over the past several decades (Brody, 1945; Bureau et al., 2002; Cho et al., 1982; Dumas et al., 2008; Kleiber, 1961).

Traditional bioenergetic systems are factorial; i.e. total energy requirements are calculated as the sum of energy required for maintenance, activity, growth, reproduction etc. (Baldwin and Sainz, 1995). The partitioning and quantification of dietary energy is important in the study of nutritional energetics because it provides a convenient platform to predict the energy balance

of individuals based on body weight, sex, activity, physiological state, environment, and amount and nutritive value of the feed eaten (Baldwin and Bywater, 1984). This information can then form the basis for practical diet formulation and evaluation (Baldwin and Bywater, 1984; Bureau et al., 2002). It is important to recognise that the factorial method is empirical in form; models based on the digestion, metabolism and utilisation of nutrients need to be considered in the context of relevant culture conditions to accurately predict growth and feed requirements.

It is recognised that the bioenergetic approach has its limitations; most notably the presumption of additivity of functions (factors) without interaction (Baldwin and Sainz, 1995) and the fact that animals continue to deposit protein while losing lipids when fed maintenance levels of digestible energy (DE) (Bureau et al., 2002; Sandberg et al., 2005; van Milgen and Noblet, 2003). There are indications that some bioenergetic models have not been well evaluated over the ranges of conditions to which they have been applied (Bajer et al., 2004), although this seems to indicate issues with the application of the models rather than the principles and fundamental concepts of bioenergetic theory. Bioenergetic models can therefore be regarded as relatively inflexible in their adaptability (Bureau et al., 2002) which is, in part, an artefact of the empirically derived nature of the sub-models. The adequacy of some feed evaluation systems has also been questioned as they are devised to meet animal requirements rather than predict animal response, which has seen a shift (back) towards nutrient-based mechanistic models to meet modern animal production demands (Dijkstra et al., 2007; Dumas et al., 2008). However some mechanistic models, while being theoretically correct, may be considered too complex for implementation in practical feed evaluation systems (Bureau et al., 2002).

In spite of these limitations, the factorial approach remains a very useful and practical method in constructing feed evaluation systems. Several models have been successfully developed to predict growth, feed requirements and feed efficiencies in a number of fish species using these principles (Cho and Bureau, 1998; Glencross, 2008; Lupatsch and Kissil, 2005; Lupatsch et al., 2001; Lupatsch et al., 1998; Zhou et al., 2005). Factorial models based on bioenergetic principles which also integrate a nutrient-based approach have the greatest flexibility and can be adapted to formulate feeds based on specific nutrient requirements (e.g. Lupatsch et al., 1998) or predict waste outputs of inorganic compounds (e.g. Hua et al., 2008). Furthermore, these types of "hybrid" models (*sensu* Dumas et al., 2008) can provide greater and more relevant application in the context of commercial production when calibrated using on-farm data (e.g. Bureau et al., 2003; Glencross, 2008; Lupatsch et al., 2003a).

The factorial modelling method for defining nutrient requirements in fish has seen advances made in recent years with the work by Lupatsch et al. (1998) and Cho and Bureau (1998). The premise behind the factorial method being that the requirements for digestible protein (DP) and DE can be partitioned into production and maintenance costs based on the assumption that the two are additive (Lupatsch and Kissil, 2005; Lupatsch et al., 2001; Lupatsch et al., 1998). This can expressed as:

Total nutrient requirement =
$$a \times BW(kg)^b + c \times Growth$$
 (1)

where a = maintenance requirement; b = weight exponent; c = utilisation coefficient

The advantage of this method over the more traditional empirical based dose response methods is that it can be used to describe DP and DE requirements for growing fish throughout the production cycle and estimations are not necessarily restricted to within the size range of the test species. Key to achieving this however are establishing the utilization efficiencies and maintenance requirements for DP and DE, an assessment of the protein and energy whole body composition as a function of fish size and establishing the growth potential under a given set of culture conditions. The requirements for DP and DE for maintenance and growth and aspects of metabolism relating to fasting and feeding physiology have been described for mulloway, *Argyrosomus japonicus* (Pirozzi and Booth, 2009a; Pirozzi and Booth, 2009b; Pirozzi et al., 2010a; 1020b); this study consolidates those published data to establish a practical feed evaluation system for this species using the factorial approach. The main objectives of this study were twofold; firstly, to use the factorial method to describe the requirements for DP and DE for mulloway up to 2 kg and, secondly, to iteratively derive diet formulations and daily feed intake based on the requirements for protein and energy. Further, this study also presents a growth model applicable over a range of temperatures relevant to Australian aquaculture conditions and also provides a quantitative description of the whole body composition of mulloway. Sensitivity analyses was used to test the response of the factorial model to small perturbations of individual parameter values on the predicted optimal ratio of DP:DE.

2. MATERIALS AND METHODS

2.1 Growth model

A data set was compiled from growth records of mulloway held at New South Wales Department of Primary Industries, Port Stephens Fisheries Institute (NSW DPI, PSFI) and a commercial mulloway farm. Farm data were based on cohorts held in sea cages or saline ponds where fish were fed to apparent satiation with commercial diets. Data from mulloway at PSFI were obtained from fish grown in 10000 l recirculating aquaculture systems or 1 m³ cages in an outdoor saline pond. Water temperatures ranged from approximately 18 - 30 °C and averaged approximately 23 °C. All growth data were expressed as mean body weight (BW g) of sub-sampled cohorts where total n > 3000 individual fish. Data outliers or cohorts where feed intake was considered spurious were excluded from the analyses. The growth model component in this study is based on body weight however workers on commercial farms often measure growth based on body length as it is a much more convenient measurement to obtain particularly if sampling from sea cages. Therefore the relationship between standard body length (SL mm) and BW was established to allow conversion from length based data to estimate BW. SL allows accurate body length measurements as it is not influenced by the condition of the caudal fin which can sometimes be damaged; however, total length (TL) is still often used. Using a range of fish from approximately 25 - 1860 g the relationship between SL and TL was also established to allow conversions based on TL. This relationship was linear and can be described as:

$$SL = 0.9428(TL) - 13.3832$$
 (r² = 0.997; n = 1072) (2)

The relationship between SL and BW was allometric (Figure 1) and can be described

BW =
$$6.163 \times 10^{-5} (SL)^{2.758}$$
 (r² = 0.99; n = 3531) (3)

2.2 Whole body composition

as:

The proportional content of energy, lipid and moisture to the BW of fish are not constant throughout the growing phase and composition also varies between species (Lupatsch et al., 2003b; Shearer, 1994). The relationship between the proximate composition and body weight of mulloway was determined using groups of equal size fish ranging from 2 - 2100 g (n = 3 to 100 fish depending on size). Samples were prepared for proximate analysis as per Pirozzi et al. (2010a; 2010b).

2.3 Dietary protein and energy utilization

The dietary protein and energy utilization efficiencies for mulloway used to populate the factorial model in this study were established in Pirozzi et al. (2010-a). Based on the slopes of regression, utilization efficiencies for DP and DE were 0.58 and 0.60 respectively. The respective corresponding cost per unit of protein or energy deposition is therefore 1.72 g DP g^{-1} and 1.67 kJ DE kJ⁻¹.

2.4 *Maintenance requirements*

The daily maintenance requirements in mulloway for energy and protein were established in Pirozzi et al. (2010-a). Maintenance requirements for energy varied depending on temperature and were 44.2 and 49.60 kJ DE kg^{-0.8} day⁻¹ at 20 and 26 °C respectively. Routine metabolic rate (RMR) and peak postprandial MO_2 have both been shown to increase linearly with temperature in mulloway (Pirozzi and Booth, 2009a; Pirozzi and Booth, 2009b); therefore, a linear relationship with maintenance energy requirement (kJ DE kg^{-0.8} day⁻¹) and temperature was also assumed which can be expressed as 26.28+0.897T (when T = 20 to 26° C).

The daily maintenance requirement for protein was found to be independent of temperature $(20 - 26^{\circ}C)$ and has been estimated in mulloway at 0.47 g DP kg^{-0.7} day⁻¹ (Pirozzi et al., 2010a)

2.5 *Parameter sensitivity analyses*

The change in model output (i.e. the predicted ratio of DP:DE) relative to the models response for a nominal set of parameter values was calculated as:

$$S = \frac{(R_a - R_n) / R_n}{(P_a - P_n) / P_n}$$

Where S is the single parameter sensitivity, R_a and R_n are the models response to altered and nominal parameter values respectively, and P_a and P_n are the altered and nominal parameters respectively (Haefner, 2005). Altered parameter values were calculated as $\pm 10\%$ of nominal values from Table 1. This method tests the influence of individual parameters and does not consider the potential multiplicative effect of the simultaneous change in two or more parameter values. Parameter sensitivity was considered at 20 °C only, although stochastic variables such as temperature can, depending on the output criteria, influence parameter sensitivity (e.g. Zhou et al., 2005).

2.6 Data analyses

Allometric relationships were iteratively derived using the non-linear least squares method in Graphpad Prism V4 (GraphPad Software, San Diego, CA, USA). All data are based on the mean of tanks or experimental units.

Mass-specific data are expressed as the geometric mean of initial and final body weights of fish (GMBW) and scaled using the metabolic body weight exponent value of 0.8 applied to energy metabolism (Clarke and Johnston, 1999; Pirozzi and Booth, 2009a; Pirozzi et al., 2010a) and 0.7 applied to protein metabolism (Glencross, 2008; Lupatsch and Kissil, 2005; Lupatsch et al., 2001).

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3. Results

3.1 Growth model

Figure 2 shows the allometric relationship between growth rate and BW of mulloway held at an average temperature of 23.6 °C (SD \pm 2.5 °C). This can be expressed as a function of temperature (*T*) within the temperature range sampled (~18 to 30 °C):

Gain (g fish⁻¹ day⁻¹) = 0.03344 × BW(g)^{0.5699} × exp^(0.0451 × T)
(
$$r^2 = 0.77$$
; n = 44 groups) (4)

Eqn. (4) can also be expressed in terms of predicted BW based on initial weight (BW_0) after time (*t*) in days as:

$$BW = (BW_0^{0.4301} + 0.0144 \times exp^{0.0451xT} \times t)^{2.3248}$$
(5)

3.2 Whole body composition

The whole body composition of mulloway (n = 45 groups) can be seen in Figure 3. Average whole body protein (19.13 g 100 g⁻¹) and ash (5.2 g 100 g⁻¹) content remained relatively constant independent of fish BW while energy, lipid and moisture demonstrated an allometric response:

Energy $(kJ g^{-1}) = 4.492 \times BW(g)^{0.0729}$	$(r^2 = 0.75)$	(6)
Lipid (g 100 g ⁻¹) = $2.063 \times BW(g)^{0.1838}$	$(r^2 = 0.53)$	(7)
Moisture (g 100 g ⁻¹) = $77.80 \times BW(g)^{-0.02}$	$(r^2 = 0.73)$	(8)

3.3 Protein and energy requirements

A summary of the parameters used to populate the factorial model are presented in Table 1. From Eqn. (1) the total requirement of mulloway for dietary protein can be described as:

DP requirement (g fish⁻¹ day⁻¹) =
$$0.47 \times BW(kg)^{0.7} + 1.72 \times protein gain$$
 (9)

and for dietary energy as:

DE requirement (kJ fish⁻¹ day⁻¹)
=
$$(26.28+0.897T)^{\times}$$
 BW(kg)^{0.8} + 1.67[×] energy gain (10)

From Eqns. (9) and (10) the total daily protein and energy requirements can then be calculated for the production range of mulloway up to 2 kg (Table 2).

3.4 Feed formulations and practical diet assignment

Based on the protein and energy requirements calculated in Table 2 the theoretical feed intake and feed conversion ratio's (FCR'S) can then be predicted for feeds with a pre specified energy content for any size mulloway up to 2 kg (Figure 4). Figure 4 is based on the "ideal" DP:DE requirement at each body weight which in practice would require many different diets with a DP:DE content to reflect this shifting requirement. Piecewise regression analyses identified significant changes in DP:DE requirement at 111, 582 and 1120 g (Figure 5). Practical feed formulations based on 4 growth stages, each with a fixed DP:DE content, are presented in Table 3.

3.5 *Parameter sensitivity analyses*

Results of the parameter sensitivity analyses are presented in Table 4. The individual parameters which have the greatest influence on the predicted requirement for DP:DE for mulloway up to 2 kg are the protein and energy utilisation coefficients and the whole body composition coefficients for protein and energy while the growth model exponent value becomes increasingly influential for fish >200 g.

4. **DISCUSSION**

4.1 Feed formulation and feed requirements

This study applied a factorial approach to quantifying protein and energy requirements for mulloway using previously published data relating to protein and energy utilization efficiencies and protein and energy requirements for maintenance (Pirozzi et al., 2010a) combined with whole body compositional and growth data. Practical diet formulations and feeding regimes for mulloway were then derived based on these predicted requirements for DP and DE. Estimates of 25.9 and 23.2 g DP MJ DE⁻¹ for a 70 and 200 g fish respectively at 26 °C using the current factorial modelling method fall close to those ranges established for mulloway using a more traditional empirically based dose response method (Pirozzi et al., 2010b). Comparison of DP:DE values between these two independent studies, which used different methodologies to arrive at similar values, appear to mutually validate the estimations of protein and energy requirements for this species.

The assignment of different diets with appropriate DP:DE content at key growth stages throughout the production cycle will assist in maximizing growth potential in mulloway. At each successive designated growth stage the DP:DE content will decrease as indicated in Table 2. Piecewise polynomial analysis (Figure 5) specified key growth stages although, for practical purposes, we can consider 100, 500 and 1100 g to represent appropriate BW indicators at which point to change diets for mulloway in commercial culture. Although the relative demand for DE increases with increasing BW there may, however, be little scope to supplement diets with non-protein energy sources as mulloway have been shown to have a limited capacity to spare dietary protein (Pirozzi et al., 2010b). The potential for mulloway to utilize non-fishmeal based protein sources and non-protein based energy sources requires further investigation.

Diets in Table 3 are presented at three different energetic contents to accommodate feeding smaller fish a low energy 15 MJ diet and larger fish with higher energy 19 MJ diets. This is necessary firstly because, on a relative basis, smaller fish consume more feed than larger fish and issues of inadequate nutrient intake may occur in larger fish unable to ingest adequate feed volumes to meet their nutrient requirements. As the requirement for DP:DE decreases with increasing fish size so to does the maximum capacity for voluntary relative feed intake (Figure 4). Secondly, to maintain an appropriate DP:DE content high energy diets require a proportionately high protein content and this may be impractical to make particularly with, for example, 19 MJ diets containing 595 g DP kg⁻¹ as indicated in Table 3.

4.2 Whole body protein and energy composition

The DP:DE requirements derived using the factorial method (Table 2) show mulloway to have a relatively high requirement for dietary protein not dissimilar to that established for white grouper (*Epinephelus aeneus*) (Lupatsch & Kissil, 2005) and barramundi (*Lates calcarifer*) (Glencross, 2008) although greater than that required by gilthead seabream (*Sparus aurata*) (Lupatsch et al., 2003c) and Euorpean sea bass (*Dicentrarchus labrax*) (Lupatsch et al., 2001). While protein composition tends to remain fairly constant between fish species, energy composition can vary considerably and also varies with body weight. The reason for the above differences seen in DP:DE requirements between species is largely due

to the different requirements for energy. It would therefore appear prudent to calibrate compositional estimations of mulloway with more fish samples >500 g as these may be underrepresented in this study (Figure 3). This will assist in refining the energy compositional model presented in Eqn. (6), and, in turn, improve the predictive accuracy of the factorial model in estimating DP and DE requirements.

4.3 Growth model

The growth model presented in Eqn. (4) is based on the growth assessment of several cohorts of fish representing the growth potential of mulloway over a range of temperatures. Care was taken to exclude cohorts performing poorly where feed intake was dubious and any outliers were also removed from the data set to ensure that the model represented the growth potential of mulloway under the given culture conditions. However, the diets fed to mulloway, also currently used by industry, may not provide an optimal DP:DE content particularly for smaller fish <500 g. Growth assessments using diets formulated according to Table 3 will allow further refinement of the growth model. Although estimations in Table 2 fall close to those DP:DE requirements estimated by Pirozzi et al. (2010b) , increasing the value of the coefficient in Eqn. (4) will in turn increase estimations in the relative demand for dietary protein (Eqn. (9)) pushing estimates even closer to those values established in Pirozzi et al. (2010b). It should also be noted that the growth model presented is relevant for temperatures ranging from ~18 – 30 °C and care should be taken when extrapolating outside these ranges.

The growth model also provides a very useful management tool to ascertain if general husbandry and feeding practices are of an adequate standard by comparing actual vs. predicted growth rates. Growth rates found to be well below those predicted in Eqn. (4) could indicate problems associated with feed intake such as the quality and/or quantity of feed offered, poor water quality, inappropriate stocking densities (see Pirozzi et al., 2009) or any number of other issues which can potentially retard growth.

Model parameters which respond allometrically with body weight require a substantial data set over which reliable predictions can be made. This is particularly the case when considering sub-models which have more than one independent variable such as the growth model presented in Eqn. (4). In this case, integrating a temperature function requires an increase in the amount of growth data by an order of magnitude representing the different levels of growth as a function of both body weight and temperature. The growth model for mulloway can therefore be further calibrated with the collation of more data over the desired production size and temperature ranges. While there are few published data on the growth rates of mulloway to compare with, particularly for fish exceeding 200 g, the model in its current form indicates a similar growth trajectory to that of barramundi and white grouper when reared at the same temperature (Lupatsch and Kissil, 2003; 2005)

4.4 Parameter sensitivity analyses

The sensitivity analyses results presented in Table 4 are insightful as they demonstrate, on several levels, the dynamic effect that small adjustments in individual parameter values have on the overall estimates for DP:DE. Several generalisations can be made. Firstly, the factorial model is fairly robust as there is very little compounding of output values with adjustments of individual model parameter values, i.e. with only minor exception, the magnitude of change in the output value was always less than the magnitude of change of the input value over the size range tested. Secondly, because the output is a ratio, an increase or decrease in any individual parameter value will directly change the output value to reflect the influence of that parameter relative to the requirement for DP. For example, an increase in protein utilisation efficiency will decrease the requirement for DP. Thirdly, the magnitude of change of the absolute output value will generally differ depending on the direction of parameter change. The exception to this is the whole body protein constant where the magnitude of change in the magnitude of change in the angitude of change in the approximation of the approximation of the influence of the approximation of the requirement for DP. Thirdly, the magnitude of change of the absolute output value will generally differ depending on the direction of parameter change.

absolute terms is equal regardless of the direction of parameter change. Lastly, the relationship of any individual parameter influence on the magnitude of change for a given body weight on the output value is allometric.

The utilization coefficients and whole body composition coefficients for protein and energy were shown to have the greatest influence. The accuracy of the utilization coefficients can be assumed with some confidence as these were determined from controlled experiments (Pirozzi et al., 2010a) and were also found to be consistent with published values for other fish species (Azevedo et al., 1998; Lupatsch et al., 2003b). The whole body composition for protein is known to remain fairly constant in fish (Shearer, 1994) and was consistent with other mulloway studies (Pirozzi et al., 2010a; 2010b). However, unlike protein composition, relative whole body energy composition will vary with body size (Figure 3) necessitating a comparatively large sample size to accurately determine whole body energy composition over the desired size range, as indicated above. Feeding history also strongly influences whole body energy composition making previous DE intake an important consideration when attempting to establish energy compositional profiles representative of a "normally" feeding population. This also has implications for any compositional analyses requiring a comparative assessment of initial and treatment samples.

4.5 Industry implications

The current practice by commercial farmers of feeding mulloway feeds formulated for barramundi or more generic "marine fish" formulations may not be ideal particularly for fish <500 g if growth rates are to be maximized. This is because some commercial feeds can typically contain 21.4 g DP MJ DE⁻¹ (e.g. Pirozzi et al., 2010a) which, when considering Table 2 may not provide an adequate proportion of DP:DE for rapidly growing smaller fish particularly if fish were fed restrictively (Pirozzi et al., 2010a; 2010b).

Dietary protein, particularly in the form of fishmeal, is usually the main driver of aquafeed ingredient costs. Therefore diets formulated as suggested in Table 3 will be more expensive than some of the less nutrient dense diets currently available. However, a diet which is optimised to match the nutritional requirements of a species will promote faster growth and improved feed conversion ratios. The cost of feeds also represents the major expense associated with running aquaculture farms; therefore the economic returns on a reduced time to market and improved FCR's need to be carefully considered when making decisions about the most appropriate feeds and feeding regimes to use. A low cost feed does not necessarily mean that it will be cost effective.

More efficient feeds, i.e. feeds that are better utilised, combined with better feeding practices will also help mitigate environmental impacts in sea cage operations by reducing excess excretion and feed wastage. This is particularly important in oligotrophic environments where excessive nutrient loading from intensive aquaculture may, for example, cause a shift in the diversity and abundance of algal assemblages in near-shore natural systems (Mannino & Sara, 2008), inturn impacting on local faunal communities.

While mulloway are a eurythermal species, a temperature of around 26 °C is likely to be the most suitable to optimise growth (Pirozzi & Booth, 2009a). Currently many commercial sites in Australia are located where mean annual water temperatures are below 20°C. At these established sites growth rates may be improved by the use of more nutrient efficient feeds and improved feeding regimes. However, if optimised diets and feeding regimes are used in combination with grow out at sites or facilities at or near optimal temperatures then the time to market will be significantly reduced. The impact of temperature on growth rates and subsequent time to market is clearly illustrated in Figure 6. From Eqn. (5), the difference between the time taken for mulloway (BW₀ = 1 g) to reach 2 kg when exposed to different temperature profiles at Port Lincoln, SA compared to Kurnell, NSW will be approximately

100 days. It should be noted that, apart from temperature, Figure 6 assumes the same set of rearing conditions, water quality, diet and feeding regimes.

5. CONCLUSION

While the predicted requirements for DP and DE determined using the factorial modeling method were validated against published data for this species, the suggested feed formulations and feeding strategies presented in this study are theoretical and remain to be tested under commercial culture conditions. Successful validation through a series of feeding trials performed under commercial culture conditions will assist in the decision by the mulloway aquaculture industry in Australia to adopt the suggested feed formulations and feeding strategies.

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Summary of parameter values used to populate the factorial model. Growth model determined at temperatures 18-30 °C, Utilization efficiencies and maintenance requirements at 20-26 °C.

Term	Equation / value
Growth rate (g fish ⁻¹ day ⁻¹) ^a	$0.03344BW^{0.5699} \times exp(0.0451 \times T)$
Whole body composition (energy) (kJ/g) ^a	$4.492 BW^{0.07288}$
Whole body composition (protein) (g/kg) ^a	191.27
Utilization efficiency (energy) ^b	0.60
Utilization efficiency (protein) ^b	0.58
Maintenance requirement DE (kJ kg ^{-0.8} day ⁻¹) ^b	26.28+0.897 <i>T</i>
Maintenance requirement DP (g kg ^{-0.7} day ⁻¹) ^b	0.47

^a Data derived from current study

^b Data derived from Pirozzi et al. (in press-a)

	Live weight g	10	50	100	200	500	800	11(
	$MBW (kg^{-0.8})^{a}$	0.03	0.09	0.16	0.28	0.57	0.84	1.0
Temperature	$\mathbf{MBW} (\mathbf{kg}^{-0.7})^{\mathbf{a}}$	0.04	0.12	0.20	0.32	0.62	0.86	1.0
20 °C	Growth (g fish ⁻¹ day ⁻¹) ^b	0.31	0.77	1.14	1.69	2.85	3.72	4.4
	DE Maintenance (kJ fish ⁻¹ day ⁻¹) ^c	1.11	4.02	7.00	12.19	25.38	36.96	47.
	Energy gain (kJ fish ⁻¹ day ⁻¹) ^d	1.63	4.58	7.15	11.16	20.11	27.20	33.
	DE growth (kJ fish ⁻¹ day ⁻¹) ^e	2.71	7.63	11.91	18.60	33.51	45.33	55.
	DE total (kJ fish ⁻¹ day ⁻¹) ^f	3.82	11.65	18.91	30.79	58.89	82.30	103
	%DE for maintenance	29.04	34.52	37.02	39.60	43.09	44.91	46.
	DP Maintenance (g fish ⁻¹ day ⁻¹) ^g	0.02	0.06	0.09	0.15	0.29	0.40	0.5
	Protein gain (g fish ⁻¹ day ⁻¹) ^h	0.06	0.15	0.22	0.32	0.54	0.71	0.8
	DP growth (g fish ⁻¹ day ⁻¹) ⁱ	0.10	0.25	0.38	0.56	0.94	1.23	1.4
	DP total (g fish ⁻¹ day ⁻¹) ^j	0.12	0.31	0.47	0.71	1.23	1.63	1.9
	%DP for maintenance	15.58	18.53	19.93	21.41	23.49	24.60	25.
	DP:DE (g DP MJ DE ⁻¹) ^k	31.30	26.62	24.77	23.01	20.83	19.77	19.
26 °C	Growth (g fish ⁻¹ day ⁻¹) ^b	0.40	1.00	1.49	2.21	3.73	4.88	5.8
	DE Maintenance (kJ fish ⁻¹ day ⁻¹) ^c	1.25	4.51	7.86	13.68	28.48	41.47	53.
	Energy gain (kJ fish ⁻¹ day ⁻¹) ^d	2.13	6.00	9.37	14.63	26.36	35.66	43.
	DE growth (kJ fish ⁻¹ day ⁻¹) ^e	3.55	10.00	15.62	24.38	43.93	59.43	72.
	DE total (kJ fish ⁻¹ day ⁻¹) ^f	4.80	14.51	23.47	38.06	72.41	100.90	126
	%DE for maintenance	25.95	31.09	33.48	35.95	39.33	41.10	42.
	DP Maintenance (g fish ⁻¹ day ⁻¹) ^g	0.02	0.06	0.09	0.15	0.29	0.40	0.5
	Protein gain (g fish ⁻¹ day ⁻¹) ^h	0.08	0.19	0.29	0.42	0.71	0.93	1.1
	DD anouth (a fak-1 dau-1)i	0.12	0.22	0.40	0 72	1 77	1 2 1	1 0

TABLE 2 Energy and protein requirements for mulloway at 20 and 26°C.

Iteratively derived feed formulations and feeding regimes at 20 and 26 °C. Estimates derived from fixed DP:DE ratios over 4 growth stages; 10-100 g = 31.3 g DP MJ DE⁻¹, 100-500 g = 24.8 g DP MJ DE⁻¹, 500-1100 g = 20.8 g DP MJ DE⁻¹, 1100-2000 g = 19.1 g DP MJ DE⁻¹. Suggested appropriate diet specifications and feeding regimes shaded in boxes.

			Live weight (g)					
	10	50	100	200	500	800	1100	2000
DE content 15.0 MJ kg	1							
DP content (g kg ⁻¹) ^a 20 °C	469.5	469.5	371.5	371.5	312.4	312.4	286.2	286.2
Intake (g fish ⁻¹ day ⁻¹) ^b	0.25	0.78	1.26	2.05	3.93	5.49	6.89	10.58
Intake (%BW day ⁻¹)	2.55	1.55	1.26	1.03	0.79	0.69	0.63	0.53
Expected FCR ^c	0.83	1.01	1.11	1.22	1.38	1.47	1.54	1.69
26 °C								
Intake (g fish ⁻¹ day ⁻¹) ^b	0.32	0.97	1.56	2.54	4.83	6.73	8.43	12.89
Intake (%BW day ⁻¹)	3.20	1.94	1.56	1.27	0.97	0.84	0.77	0.64
Expected FCR ^c	0.80	0.96	1.05	1.15	1.29	1.38	1.44	1.57
DE content 17.0 MJ kg ⁻¹								
DP content (g kg ⁻¹) ^a 20 °C	532.1	532.1	421.0	421.0	354.1	354.1	324.3	324.3
Intake (g fish ⁻¹ day ⁻¹) ^b	0.22	0.69	1.11	1.81	3.46	4.84	6.08	9.33
Intake (%BW day ⁻¹)	2.25	1.37	1.11	0.91	0.69	0.61	0.55	0.47
Expected FCR ^c 26 °C	0.73	0.89	0.98	1.07	1.22	1.30	1.36	1.49
Intake (g fish ⁻¹ day ⁻¹) ^b	0.28	0.85	1.38	2.24	4.26	5.94	7.44	11.38
Intake (%BW day ⁻¹)	2.82	1.71	1.38	1.12	0.85	0.74	0.68	0.57
Expected FCR ^c	0.70	0.85	0.93	1.01	1.14	1.22	1.27	1.38
DE content 19.0 MJ kg ⁻¹								
DP content (g kg ⁻¹) ^a 20 °C	594.7	594.7	470.6	470.6	395.7	395.7	362.5	362.5
Intake (g fish ⁻¹ day ⁻¹) ^b	0.20	0.61	1.00	1.62	3.10	4.33	5.44	8.35
Intake (%BW day ⁻¹)	2.01	1.23	1.00	0.81	0.62	0.54	0.49	0.42
Expected FCR ^c 26 °C	0.66	0.80	0.88	0.96	1.09	1.16	1.22	1.33
Intake (g fish ⁻¹ day ⁻¹) ^b	0.25	0.76	1.24	2.00	3.81	5.31	6.65	10.18
Intake (%BW day ⁻¹)	2.53	1.53	1.24	1.00	0.76	0.66	0.60	0.51
Expected FCR ^c	0.63	0.76	0.83	0.91	1.02	1.09	1.14	1.24

^aDP content = Fixed DP:DE (values noted in Table 6.4 caption) x DE content

^b Intake = DE total (from Table 6.3) / DE content

^c Expected FCR = Intake / Growth (from Table 6.3)

Parameter sensitivity analysis. Values represent % change in the predicted DP:DE values at 20 °C (Table 2) after altering individual model parameter values $\pm 10\%$. Refer to Table 1 for original individual model parameter values. Parameters shown ranked in order of greatest to least influence on predicted DP:DE requirement based on the average (absolute) value over the fish weight range shown.

	Live fish body weight (g)									
Altered										
value	Parameter	10	50	100	200	500	800	1100	2000	Average
+10%	Whole body composition constant (protein)	8.4	8.1	8.0	7.9	7.7	7.5	7.5	7.3	7.8
	Utilisation efficiency coefficient (protein)	-7.9	-7.6	-7.5	-7.4	-7.2	-7.1	-7.0	-6.9	-7.3
	Growth weight exponent	1.7	3.4	4.3	5.3	6.7	7.5	8.1	9.2	5.8
	Utilisation efficiency coefficient (energy)	6.9	6.3	6.1	5.8	5.5	5.3	5.1	4.9	5.7
	Whole body composition coefficient (energy)	-6.6	-6.1	-5.9	-5.7	-5.4	-5.2	-5.1	-4.9	-5.6
	Metabolic weight exponent (energy)	9.8	7.9	6.6	5.0	2.4	0.8	-0.4	-2.7	4.5
	Maintenance constant (energy)	-2.8	-3.3	-3.6	-3.8	-4.1	-4.3	-4.4	-4.6	-3.9
	Maintenance constant (protein)	1.7	2.1	2.2	2.4	2.6	2.7	2.8	3.0	2.4
	Whole body composition exponent (energy)	-1.2	-1.9	-2.1	-2.3	-2.6	-2.7	-2.7	-2.8	-2.3
	Metabolic weight exponent (protein)	-4.3	-3.5	-3.0	-2.3	-1.1	-0.4	0.2	1.3	-2.0
	Growth coefficient	1.3	1.5	1.6	1.7	1.9	1.9	2.0	2.1	1.7
	Growth temperature exponent	1.2	1.4	1.5	1.6	1.8	1.8	1.9	1.9	1.6
-10%	Utilisation efficiency coefficient (protein)	9.7	9.4	9.2	9.1	8.8	8.7	8.6	8.4	9.0
	Whole body composition constant (protein)	-8.4	-8.1	-8.0	-7.9	-7.7	-7.5	-7.5	-7.3	-7.8
	Whole body composition coefficient (energy)	7.6	7.0	6.7	6.4	6.0	5.8	5.7	5.4	6.3
	Utilisation efficiency coefficient (energy)	-7.3	-6.8	-6.5	-6.3	-5.9	-5.8	-5.6	-5.4	-6.2
	Growth weight exponent	-1.8	-3.7	-4.6	-5.6	-7.0	-7.8	-8.3	-9.3	-6.0
	Metabolic weight exponent (energy)	-11.5	-8.5	-7.0	-5.2	-2.4	-0.8	0.4	2.7	-4.8
	Maintenance constant (energy)	3.0	3.6	3.8	4.1	4.5	4.7	4.8	5.1	4.2
	Metabolic weight exponent (protein)	5.9	4.3	3.5	2.6	1.2	0.4	-0.2	-1.3	2.4
	Whole body composition exponent (energy)	1.2	1.9	2.1	2.3	2.6	2.7	2.8	2.9	2.3
	Maintenance constant (protein)	-1.6	-1.9	-2.0	-2.2	-2.4	-2.5	-2.6	-2.8	-2.3
	Growth coefficient	-1.4	-1.7	-1.8	-1.9	-2.1	-2.1	-2.2	-2.3	-2.0
	Growth temperature exponent	-12	-1.5	-16	-17	-18	-18	-19	-2.0	-1.7

Note: Ranked average values are for illustrative purposes and will obviously change depending on nominated body weight and range.



Relationship between standard length (mm) and body weight (g) of mulloway. Weight measurements range from 12 - 1600 g. ($r^2 = 0.99$; n = 3531).



Relationship between BW (g) and growth rate (g fish⁻¹ day⁻¹) of mulloway held at an average temperature of approximately 23 °C (solid line). Data points represent mean values of groups of fish (n = 44). Dashed lines represent estimations of growth rates at the lower and upper ranges of temperatures occurring during growth trials (18 – 30 °C) based on Eqn. (4).



Relationship between proximate body composition and live weight (g) (n = 45 groups). Diamonds = moisture; Circles = energy; Triangles = protein; Crosses = lipid; Squares = ash.



Relationship between theoretical FCR and feed intake values (%BW) and BW for mulloway fed diets with three different DE contents (15, 17 or 19 MJ kg⁻¹). Predicted FCR's increase with increasing BW, feed intake as a proportion of BW decreases with increasing BW. Values based on theoretical feed intake at 26 °C with diets optimized for decreasing DP:DE demands with increasing BW.



Theoretical requirement for DP:DE ratio at 20 to 26°C. Breakpoints (dashed vertical lines) derived from piecewise analysis occur at 111, 582 and 1120 g.



Predicted growth rates of 1g mulloway to 2 kg at two different site locations in Australia. Growth rates calculated from Eqn. (5) based on 2005 - 07 daily sea surface temperatures (SST) (secondary y-axis) at Port Lincoln, SA (dotted lines) and Kurnell, NSW (solid lines). SST source: Bureau of Meteorology, Australia.

4.11 Effect of winter or summer water temperatures on weight gain, feed intake and feed conversion ratio of juvenile yellowtail kingfish *Seriola lalandi* fed a range of different commercial feeds

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ABSTRACT

A 42 day study was undertaken to examine the effect of water temperature (16°C and 23°C) on the weight gain and performance of yellowtail kingfish Seriola lalandi fed six commercially manufactured diets (Winter, GMO-LAP Free, Marine 90238, Marine 90239, Marine 90237, Marine 90235). Diets were similar in crude protein (460 g kg⁻¹), lipid (200 g kg⁻¹) and gross energy (22 MJ kg⁻¹) content, but the type and ratio of lipids used in each diet was different (i.e. fish oil or poultry oil) and the level of fishmeal in diets varied between 35% and 50% (commercial in confidence). Each of the 12 treatments was assigned to 3 replicate 200L cages housed within larger 10 kL tanks connected to two separate saltwater recirculating systems. Each replicate cage was stocked with 5 juvenile fish (mean±sd stock weight = 180 ± 17 g). All fish were fed twice daily to apparent satiation Monday to Friday (0900h and 1400h) and only once on Saturday and Sunday (0900h). Due to the separate thermal regimes, response data on relative weight gain, relative feed intake, FCR and thermal growth coefficient (TGC) were analysed using a classic multi factor split-plot design. Results of multifactor ANOVA indicated that temperature but not diet type nor the interaction of terms had a significant positive affect on weight gain, feed intake, FCR and TGC (all P<0.002). Elevating the rearing temperature from 16°C to 23°C more than doubled the relative weight gain of yellowtail kingfish (10.6g kgBW d^{-1} vs 22.5 g kgBW d^{-1} ; n=6), increased relative feed intake by approximately 53% (22.28 g kgBW d⁻¹ vs 34.25g kgBW⁻¹ d⁻¹; n=6) and improved feed conversion ratio from 2.16 to 1.57. The thermal growth coefficient pooled across diets (n=18) was determined to be 1.34 and 2.07 for fish reared at 16°C or 23°C, respectively. These results demonstrate overwhelmingly that the aquaculture production of yellowtail kingfish will be far more economical at temperatures approaching 23°C. It has also demonstrated that substitution of fish oil with different levels of poultry oil or feeding diets that contain different levels of fish meal do not adversely affect the short term performance of vellowtail kingfish reared at either of these temperatures. Assuming a constant water temperature of 16°C and a stocking size of 5g, yellowtail kingfish would take approximately 508 days to reach 2 kg. By contrast, if a water temperature of 23°C was maintained they could expect to reach the same body weight in approximately 229 days.

1. INTRODUCTION

In 2004 the production of all cultured *Seriola spp.* in Japan was approximately 150,000 tonnes, achieving a value of nearly US \$1.34 billion (Nakada, 2008). The dominant species cultured in Japan is the Yellowtail *Seriola quinqueradiata,* with *S. dumerili, S. lalandi* and *S. aureovittata* making up the balance of production. *S. lalandi*, also known as Yellowtail kingfish or gold striped amberjack is also cultured in Australia, New Zealand and Chile (Moran, Pether & Lee, 2009). Aquaculture of *S. lalandi* in Australia is mostly conducted in the Eyre Peninsula region of South Australia (SA) at Fitzgerald Bay, Cowell and Port Lincoln (Rimmer & Ponia, 2007). In South Australia, fingerlings are cultured in sea cages from 5 g onwards and they are usually stocked in October in order to gain maximum exposure to the increasing water temperatures associated with the onset of summer (Fernandes & Tanner, 2008). Under this scenario they typically reach a body weight of 2-3 kg in 12 to 15 months.

The farming of S. lalandi in SA faces many challenges, but key amongst them is the impact of low winter temperatures on feed intake and growth and the combined effect these factors have on whole of farm production strategies and efficiencies. Seasonal sea water temperature at South Australian (SA) sites varies considerably. During summer, water temperatures can reach 24°C which is also typical of the water temperatures reached in summer on the eastcoast of NSW (Figure 1). During the winter season water temperature in some locations in SA can drop to 13°C. At these temperatures yellowtail kingfish are reported to either stop growing or show markedly reduced growth rate (Kolkovski & Sakakura, 2004; Kolkovski, 2005). Recent research has suggested that their optimal water temperature is 22.8°C (Pirozzi & Booth, 2009). One reason for the decline in growth rate may be a decrease in metabolic rate or a decrease in energy requirement due to reduced physiological activity (Watanabe, Kuriyama, Satoh, Kiron, Satoh & Watanabe, 2001). Associated with low winter temperatures and reduced growth potential is the concomitant and dramatic decline in feed intake. These declines have led to elevated feed conversion ratio (FCR), especially in fish approaching their second winter of grow-out. Whether these elevated FCR's are a consequence of physiological processes or a result of sub-optimal feeds of feeding regimes is unclear at the present time. Winter performance of Seriola spp's has been the focus of considerable research in Japan for many years (Nakada, 2001). And as such these issues are not unique to the Australian kingfish industry.

To date there is no published information on the nutritional requirements of *S. lalandi*. However, there are many publications on related species such as *S. quinqueradiata* that suggest *Seriola spp*. have a high requirement for dietary protein (>50%) due to their increased dependence on this substrate for both metabolic protein and energy requirements (Moran et al., 2009). Whether or not these gross requirements are similar in *S. lalandi* is still to be determined, however *S. lalandi* have a far wider geographical distribution and naturally grow to larger sizes than *S. quinqueradiata* (Nakada, 2008), which may indicate they are dissimilar in at least some of their nutritional and physiological requirements.

In Australia, S. lalandi are exclusively fed extruded pellets which are supplied by a small number of feed manufacturers. Each manufacturer provides a range of diets formulated for different sized fish as well as for different temperature regimes. The nutrient and energy level, ingredient composition, level of supplements and attractants, bulk density and the sophistication of feeding regimes used to deliver these feeds will invariably affect production characteristics. As elsewhere, these factors are inextricably linked to the aforementioned variability in seasonal water temperatures at different sites. Prior scientific evaluation of commercial feeds for S. lalandi has not been undertaken in Australia. Therefore the aim of this study was to evaluate the performance of S. lalandi reared at one of two temperature regimes and fed on one of six different commercially manufactured feeds. The temperature regimes were selected to reflect the lower and upper seasonal water temperatures in SA and NSW, respectively (i.e. 16 vs 23°C). The six proprietary commercial feeds were provided by one Australian feed manufacturer and differed in their ingredient, nutrient and energy composition as well as minor differences in pellet characteristics. Diets were formulated to investigate potential improvements to standard formulations for yellowtail kingfish, however these modifications are currently held "commercial in confidence".

2. MATERIALS & METHODS

2.1 Commercial diets

Fish fed each of six commercial diets were compared at each temperature (16°C or 23°C). Six diets were provided by one commercial manufacturer. These diets were formulated to have similar crude protein, gross energy and fat levels, but diets varied in type of oil, oil mixture and oil ratios and ingredients. The manufacturer supplied all diets with a trade name or product run number (i.e. Winter, GMO-LAP Free, Marine 90238, Marine 90239, Marine

90237, Marine 90235) and all diets were delivered as 6mm pellets in hermetically sealed bags containing manufacturer contact details, diet descriptions and nutritional panels. Diets were transferred to clean plastic drums with tight fitting lids and stored in a freezer during the experiment (<-18°C).

2.2 Handling procedures

The yellowtail kingfish, *S. Ialandi*, used in this experiment were progeny of wild caught brood stock held at the NSW DPI Port Stephens Fisheries Institute (PSFI). Prior to the experiment juveniles were reared at low densities in large 10 kL tanks and fed once or twice daily on a high protein commercial finfish feed. Prior to stocking or weight check procedures all fish were starved for 24 hours. Fish were always handled, weighed and measured after being anaesthetised (15-25 ppm Aqui-STM). At stocking and at harvest (42 days) each fish was weighed to the nearest gram and its fork length recorded to the nearest millimetre. An intermediate weight check was performed after 21 days, however at this time only the bulk weight of fish in each cage was recorded. Moribund or dead fish were weighed and replaced with fish of equal weight, however, one fish from a replicate cage assigned to diet 3 died 10 days before the experiment was terminated and this fish was not replaced. At the completion of the experiment all fish were returned to large holding tanks.

2.3 Experimental systems

The growth experiment was done in two separate saltwater recirculation systems (RAS) at PSFI that allowed two different temperature regimes to be established. Each RAS consisted of 3 x 10kL circular fibreglass tanks (3.4m diameter x 1.2m depth) located inside a plastic covered shade house. Each system was connected to a large sump tank which collected returning effluent water that was continuously pumped through a foam fractionating column or rotating-bed biological filter, respectively. Water was pumped from the collecting sump and returned to each of the larger tanks through a large sand filter. Influent rates at each tank were set at approximately 60 L min⁻¹. Approximately 10-15% of the effluent was discarded every day either through vacuuming to remove accumulated faecal material or back-flushing the sand-filters. Losses were replaced by using clean disinfected estuarine water from a separate reservoir system. Each 10kL tank was constantly aerated with large submerged diffusers. In addition tanks connected to the high temperature system were supplied with oxygen to ensure levels of dissolved oxygen remained above 6 mgL⁻¹.

The inner-most lining of the shade house was lined with black shade-cloth to prevent the proliferation of algae and provide subdued light conditions during daylight hours. Otherwise the trial was run under the prevailing winter light conditions between 14.7.09 to 25.8.09 (approximately 12L:12D). Each of the large tanks contained $6 \times 0.2m^3$ cylindrical experiment cages (0.6m diameter x 0.7m submerged depth) made of 10mm perforated plastic mesh. Each cage was fitted with a plastic lid (1.6mm plastic mesh) to prevent fish from escaping. Experiment cages were secured an equal distance apart around the outer perimeter of each 10kL tank and remained in the same position during the entire experiment. Thus a total of 36 cages were deployed in this experiment, 18 per RAS system.

Water quality was measured daily using a calibrated electronic water quality instrument (Horiba U-10). Total ammonia nitrogen (TAN) was measured using a rapid test kit procedure (Aquamerck). During the experiment mean±sem of pH, salinity and dissolved oxygen were 7.8±0.1 units, $30.5\pm1.6\%$, 8.2 ± 1.1 mg L⁻¹. Total ammonia was always ≤ 1.0 mg L⁻¹. The temperature of each system was continuously logged every 3 hours using separate submerged data recorders (Tinytag TG 4100 (-40°C to +85°C, Gemini Data Loggers (UK) Ltd). The mean±sem of the low temperature system was $16.7\pm0.9^{\circ}$ C with a modal temperature of 16.0°C. The mean±sem of the high temperature system was $23.0\pm0.7^{\circ}$ C with a modal temperature of 23.0°C.

2.4 Stocking and acclimation procedure

Prior to the experiment, fish were housed in a separate RAS and held at a temperature of 22-23°C. As a consequence and prior to stocking procedures, each of the experimental systems was initially warmed to a similar temperature using 2400kW immersion heaters. After fish were stocked into the experiment the immersion heaters were disconnected from the cool water system which allowed the water temperature to gradually decline over a period of 4-5 days.

Each experiment cage was stocked by randomly selecting fish from the population of fish held in larger holding tanks. Fish were prior graded to the desired weight range and to exclude animals with minor deformities. Each cage was systematically stocked with 5 fish having a mean \pm sem individual weight of 180 \pm 17g (n=180). Fish were given a small acclimation feed following stocking procedures and switched to test feeds the following day. Fish were carefully fed to apparent satiation twice daily Monday to Friday (i.e. 0900h and 1400h) and once daily on Saturday and Sunday (0900h).

2.5 Chemical analyses

Each of the commercial diets was analysed for dry matter (DM), crude protein (Nx6.25), fat (ether extract), ash and gross energy (bomb calorimeter) by the Queensland Department of Primary Industries & Fisheries (QDPI&F) Animal Research Institute - Health and Nutritional Biochemistry Laboratory, Moorooka, Qld, Australia using specific in-house or standard AOAC (1990) procedures. The "as received" nutrient and energy composition of the six diets is presented in Table 1.

2.6 Statistical analyses

The experiment was designed for analysis using a classic multifactor split-plot *ANOVA* design (Quinn & Keogh, 2002). Fixed factors were water temperature (2 levels; 16 vs 23° C) and diet type (6 levels). Tank number was considered random and nested within temperature regime. Each of the 12 treatments was replicated in 3 experiment cages, with each test diet randomly allocated to one cage within each tank. Data were checked for homogeneity of variance (Cochran's test) prior to *ANOVA* and treatment means were separated using Tukey-Kramer multiple comparisons test. Alpha for all tests was set *a priori* 0.05 and data were analysed using the general linear model function in NCSS version 2004.

The major response variables of interest were relative weight gain, relative feed intake, and feed conversion ratio (FCR). The thermal growth coefficient (TGC) of fish reared on each diet at each temperature was also calculated for comparative and predictive purposes.

The following equations were used to derive the average performance variables for fish in each cage:

Relative weight gain (g kgBW d^{-1}) = individual weight gain / (GMBW/1000) / days	Eq 1.
Relative feed intake (g kgBW d^{-1}) = individual intake / (GMBW/1000) / days	Eq 2.
FCR = individual feed intake as is basis / individual wet weight gain	Eq 3.
$TGC = [(FBW^{0.3333} - IBW^{0.3333}) / (T \times D)] \times 1000,$	Eq 4.

where GMBW = geometric mean body weight, FBW = final body weight, IBW = initial body weight, T = temperature, D = days.

3. **RESULTS**

Maintenance of the two different temperature regimes under ambient winter conditions was reasonably successful as indicated by the stability of the water temperature in either system (Figure 2). Annual ambient winter air temperatures in NSW in 2009 were the highest on record and this phenomenon tended to elevate temperatures in the cool water regime towards the end of the experiment. Notwithstanding this event, the majority of the experiment was conducted at the pre-planned temperatures of 16 and 23°C.

Feeding activity of kingfish was noticeably reduced at 16° C compared to the activity of fish reared at 23°C. This reduction in activity was even noted during the brief acclimation phase. Overall, there was a strong effect of water temperature on feed intake and growth potential, with all measures of performance being dramatically reduced at the low water temperature. Despite the reduced rate of growth and feed intake at lower temperature there was no mortality associated with this regime. Three fish died during the experiment but these mortalities were not related to any particular diet. Fish reared in the high temperature regime grew rapidly, more than doubling their body weight over the course of the experiment. Data on individual treatment mean \pm sem for stock weight, harvest weight, absolute feed intake, FCR, relative weight gain, relative feed intake and TGC are presented for cold water and warm regimes in Tables 2 and Table 3, respectively.

Multifactor *ANOVA* indicated that the effect of temperature was responsible for explaining the majority of the observed differences in each of the response variables. In addition, the effect of diet type and the interaction of diet type and temperature were non-significant in all response variables (P>0.05; Table 4). Overall, rearing fish at 23°C as opposed to 16°C more than doubled relative weight gain (10.62g kgBWd⁻¹ vs 22.52g kgBWd⁻¹). Increased rearing temperature also increased the TGC by nearly 53% and improved feeding efficiency from approximately 48% to 75% (Table 5). The higher temperature regime increased relative feed intake from 22.28g kgBWd⁻¹ to 34.15g kgBWd⁻¹. An *a priori* alpha value of 0.05 was selected to examine the effect of temperature and diet type on performance of yellowtail kingfish. However multifactor *ANOVA* indicated that the effect of diet type was significant at the 90% confidence interval. A post-hoc multiple comparisons procedure on the marginal means of diet type (n=6) using an alpha value of 0.10 indicated a significant difference between the relative weight gain, TGC and FCR of fish fed the "Marine Winter" diet and those fed the diet containing 100% fish oil or Marine CST.

4. **DISCUSSION**

The results of this study clearly demonstrate the strong influence of water temperature on the weight gain, feed intake and resultant feed conversion efficiency of juvenile yellowtail kingfish. This study has also demonstrated that the performance of yellowtail kingfish was not unduly influenced by the six commercial diets we tested, with all formulations supporting statistically similar increases in weight gain and similar levels of feed efficiency at the 95% confidence interval. Our study has also indicated that the thermal growth coefficient (TGC) for juvenile yellowtail kingfish is quite different at 16°C and 23°C, demonstrating TGC over the range of temperatures bracketed by our study is not independent of temperature (Jobling, 2003).

The impact of water temperature on the performance of *Seriola spp.* has received much attention, particularly from researchers working within the Japanese industry which dates back to the mid 1960's (Nakada, 2001). Minamisawa & Sakai (1969; cited in Kohbara, Hidaka, Matsuoka, Osada, Furukawa, Yamashita & Tabata, 2003) were perhaps the first researchers to observe the effect of temperature on feeding activity in yellowtail. Their early observations indicated that at temperatures below 11°C no feeding occurs; at 13-17°C feeding activity was observed but its frequency was low; 18-27°C was a suitable range for feeding

while 24-26°C was adjudged the best range. Feeding activity ceased above 28°C. In subtropical regions of Japan such as Okinawa, where water temperatures average 20-24°C, mojako (i.e. *S. quinqueradiata* < 200g) can reach up to 6kg within 24 months. At average water temperatures of 17-19°C they require nearly 36 months to reach the same harvest weight (Nakada, 2001). According to Nakada (2001), these regions only offer optimal water temperatures for the culture of *S. quinqueradiata* for about 75% and 50% of the year, respectively.

Recent research investigating the effects of temperature on the routine metabolic rate of juvenile S. lalandi has shown their metabolic rate (Q_{10}) is least affected when they are held at a temperature of 23°C. Consequently, and in the absence of other abiotic or biotic factors this temperature was considered optimal for rearing this species (Pirozzi & Booth, 2009). It is known that water temperature acts as a "controlling factor", regulating the metabolic requirements for food and governing the rate at which food is processed by fish species (Brett, 1979). Therefore, culture of S. lalandi at temperatures too far below or too far above this optimum will negatively impact on performance by causing a shift in the efficiency of chemical reactions related to metabolism. The implications of this are profound when considered in terms of the areas where S. lalandi are farmed in Australia. For instance, if we adopt a similar approach to that of Nakada (2001) and accept a lower temperature threshold of 19°C for the economic production of S. lalandi, then the percentage of culture days where this occurs in Kurnell (NSW), Arno Bay (SA) or Port Lincoln (SA) over a 2 year cycle is 69%, 41% or 25%, respectively (Figure 1). Unlike the Japanese experience, high water temperatures approaching 30°C are unlikely to be an issue for sea cage rearing of S. lalandi in either NSW or SA.

Based on the diets we evaluated and the average TGC's for each of our temperature regimes (i.e. 1.34 vs 2.07), yellowtail kingfish stocked at the present industry standard of 5g could be expected to reach a body weight of 2kg in approximately 508 days if reared at a constant temperature of 16°C. This could be reduced to a period of approximately 229 days if they were reared at a constant temperature of 23°C. Further, we modelled the dependence of the TGC on temperature by assuming a linear relationship between these variables over the temperature range of 16 to 23° C (Jobling, 2003). The resultant equation was (TGC = 0.1043 x water temperature - 0.3286). Derivation of this equality allowed prediction of theoretical weight gain in S. lalandi in response to changes in seasonal water temperature. Therefore, two different scenarios were modelled; the first assumed a stocking weight of 5g and the second assumed a stocking weight of 50g. Fish were stocked on the 2nd October and growth was modelled on the cyclic sea-surface temperatures recorded at Arno Bay (South Australia) by the Australian Bureau of Meteorology (BOM) between August 2005 and August 2007 (Figure 3). Assuming a harvest weight of 4kg is desirable, fish stocked at 5g would be ready to harvest around the 8th April 2007. In contrast fish stocked at 50g would be ready to harvest around the 18th February 2007, some 6-7 weeks earlier (Figure 3). This type of predictive model could be improved by scientifically evaluating the effect of a wider range of water temperatures on growth rate, TGC, feed intake and feed efficiency in S lalandi and would be an extremely useful management tool for sea-cage farming of this species in Australia.

Feed intake at each of the temperatures used in our study was slightly higher than recommendations made by Sakamoto (Sakamoto et. al. 1997 cited in Watanabe, 2001) for *S. quinqueradiata* fed soft dry pellets with an average crude protein, crude lipid and gross energy content of about 45%, 21% and 18.4 MJkg⁻¹, respectively. However, our feed intake values were within the recommended upper (2.25% BWd⁻¹) and lower (1.8%BWd⁻¹) range for 400g *S. quinqueradiata* fed dry extruded pellets with similar gross nutrient and energy content (Nakada, 2001) to the diets provided by Ridley Aquafeed Pty Ltd (Table 1). Relative feed intake in our study was higher than values recorded for *S.lalandi* reared in small tanks at similar temperatures by Moran et al. (2009). We adopted a satiated feeding regime in this study that delivered two feeds per day Monday to Friday and one meal on Saturday and

Sunday, respectively. This may have influenced the results of this study to some extent. For example, self feeding behaviour of wild *S. quinqueradiata* juveniles indicated they follow both a crepuscular (dusk and dawn) and nocturnal feeding pattern closely synchronised to the prevailing photoperiod and seasonal changes in light intensity; water temperature dictating the overall demand for feed and temporal changes in light intensity governing the pattern of feeding (Kohbara et al., 2003). Our experiment was conducted under a natural photoperiod of 12L:12D, however the intensity of natural sunlight reaching the experimental systems was attenuated by the materials covering the greenhouse and a layer of black shade-cloth. This is not likely to have overly influenced our results as the level of light intensity and duration was similar across all tanks and cages. However, it is possible that higher levels of feed intake may have been achieved had we synchronised our AM-PM feeding regime to coincide with the dawn-dusk cycle. This issue remains to be resolved for *S. lalandi*. Much evidence also exists detailing the interaction between feeding regime (e.g. 1, 2, 3 or 5 times per week) and the passage and digestion of dry extruded pellets from the stomach of *S. quinqueradiata* reared under winter conditions. These studies suggest that reduced rates of feeding slow

stomach and intestinal evacuation time in an adaptive response which probably increases digestion of pellets (Watanabe et al., 2001). On-farm feeding practices in Australian sea-cage operations vary widely depending on the season, scale of the venture, the number of staff and the available technology. However, hand feeding yellowtail kingfish on extruded pellets once or twice daily is still the dominant form of delivery.

As indicated earlier, the formulations of each feed were commercial in confidence, however fish reared on the fortified diet (i.e. Marine Winter) recorded numerically higher weight gain, numerically higher TGC and better feed conversion ratio than fish fed the other diets. This may indicate that the formulation of this feed and or the specific additives used to fortify this diet or improve its palatability might have promoted an even greater difference in weight gain had the trial continued. As stated previously, the main effect of diet was not significant at the 95% confidence interval. However, there was an approximately 20% improvement in the relative weight gain of yellowtail kingfish reared on the "Marine Winter" diet compared to fish reared on the two poorest performing diets (i.e. comparison of marginal means; n=6; Table 5). Relaxation of alpha from our *a priori* probability of 0.05 to 0.10 causes the nullhypothesis of no difference between diets to be rejected. Subsequently, the multiple comparisons procedure was able to identify a positive significant influence on the relative weight gain, TGC and FCR of fish fed the "Marine Winter" diet compared to those fed other diets. Relaxation of the alpha value increases the risk of committing a Type 1 error (falsely rejecting the null hypothesis). However, at the same time it reduces the risk of committing a Type II error (failing to detect a change that has occurred). In the search for greater efficiencies in the production of yellowtail kingfish these risks may be acceptable given the improvements recorded in kingfish fed the better performing diets examined in this trial. Obviously the decision to use any of the better performing diets would need to be considered in terms of overall economic benefit, taking into account the cost of new feeds and their expected conversion ratios over the whole production cycle.

In New Zealand, as in Australia, diets for grow-out of *S. lalandi* have been selected based on their physical properties, price and availability rather than on their ability to meet the nutritional requirements of the animal (Moran et al., 2009). Diets high in crude protein and fishmeal are thought to provide *S. lalandi* with adequate protein for growth and energy purposes, but these assumptions have not been thoroughly tested. Production results based on the feeding of commercial diets are rare, however Fernandes & Tanner (2008) recently published an environmental study at Fitzgerald Bay (SA) investigating the flow of nutrients from two sea-cages stocked with *S. lalandi* fed commercial pellets with 44% crude protein content. Feed conversions were above 3.0:1.0, but these values were for larger fish growing between 1 to 3kg in body weight under quite variable operating conditions and may not be indicative of the maximum growth and potential feed conversion obtainable by this species reared on high quality commercial feeds and appropriate feeding regimes. Moran et al. (2009)

have recently published a much more rigorous trial in which they evaluated performance of S. lalandi grown in replicate tanks and reared on 3 commercial feeds used by NZ producers of this species. Comparative data from their study has been reproduced in Table 6. The commercial feeds used in their study varied in protein and energy content, ingredient and nutrient composition and pellet characteristics. Interestingly, although their 114 day experiment was run at ambient austral water temperatures they classified each stage of the experiment into a cool water (i.e. median of 14-17°C; 47 days) period and a warm water period (i.e. 17-22°C; 67 days). These temperature categories were similar to those investigated in our study. Their fish were also fed twice daily at 0900 and 1500h. Growth rate almost doubled (i.e. TGC of ca. 1.0 vs ca. 1.9) and mass specific feed intake increased by 1.6 to 1.7 times during the warmer phase of their study. Unlike our study, these authors also found clear and significant differences between dietary treatments in both phases of their trial, but interestingly, these differences appear to be more profound at lower temperatures and for indices such as FCR, mass specific feed intake and daily energy intake, rather than for growth indices such as final weight or SGR (Moran et al., 2009). These differences were not as evident in our trial presumably due to the fact that each of the diets we tested was fairly similar in nutrient and energy content as well as physical characteristics. In addition, use of different lipid sources, lipid ratios and protein sources in the diets we investigated appear to have had little affect on performance, albeit these observations were made over a relatively short time frame. An earlier un-replicated study (20°C) in which juveniles of S. lalandi (c. 57g stock weight) were fed twice daily for 37 days on commercial diets containing different ratios of crude protein: lipid (i.e. 45:25, 54:18 or 50:12) also indicated that harvest weights of fish (30 per tank) were not different, however FCR of fish fed the high fat diet (45:25) was better (i.e. 0.88) than the FCR of fish fed the intermediate (i.e. 0.91) or low fat diet (0.93), respectively (Kolkovski, 2005). Similarly, a 118 day study that fed commercial diets which contained protein: lipid ratios of 40:18, 40:22, 40:26 or 40:30 to Mediterranean yellowtail S. *dumerilii* found that weight gain and protein efficiency ratio were not different at the end of the trial, however although not significant there was a numerical improvement in FCR with increasing levels of dietary lipid, with the 40:26 diet recording the best (lowest) FCR (Talbot, Garcia-Gomez, De La Gandara & Muraccioli, 1999).

Extruded pellets containing more than 20% fat are efficiently utilised by S. quinqueradiata and FCR's as low as 1.2 have been achieved at elevated temperatures in fish during their first year of culture (i.e. <1kgBW) (Nakada, 2001). High protein (49%) high fat diets (26%) fed to S. lalandi of about the same finishing weight have also returned FCRs between 1.22 and 1.37 (Moran et al., 2009). FCR tends to increase (worsen) with fish size and Japanese growers often switch back to feeding minced raw fish mixed with binding agents when S. quinqueradiata exceed 3kg in body weight (Nakada, 2008) or the growth rate of fish fed extruded pellets falls during the winter season (Kofuji, Akimoto, Hosokawa & Masumoto, 2005). FCR appears to be very poor in most Seriola spp. during winter or low temperature conditions and is exacerbated by inappropriate feeding regimes (Watanabe, Aoki, Yamagata, Kiron, Satoh & Watanabe, 2000; Watanabe et al., 2001). The effects of temperature on FCR in this study are extremely interesting. Poor FCR under our low temperature regime may be related to several issues. It may be possible that S. lalandi under either temperature regime were slightly over or under fed which would worsen FCR. Nakada (2002) has suggested that digestibility of dry commercial feeds fed to S. quinqueradiata may be lowered due to the buffering action of these feeds on stomach acids. For these reasons and due to reduced energy requirements the winter feeding of S. quinqueradiata has generally been reduced to 2 or 3 times per week (Watanabe, 2001). Still others have suggested that winter reductions in the amount and activity of proteases such as trypsin, chymotrypsin and pepsin in the digestive system of species such as S. quinqueradiata may lower overall apparent protein digestibility of feeds, indirectly reducing the amount of digestible protein (amino acids) available for growth purposes (Kofuji et al., 2005). Introduction of feeding stimulants such as synthetic and natural krill or squid extracts enhanced secretion of pepsin, trypsin and chymotrypsin enzymes in S. guinqueradiata and improved protein digestibility at low water temperature

(Kofuji, Hosokawa & Masumoto, 2006). The causative factors involved in the lowering of FCR in *S lalandi* reared at low water temperatures and the identification of suitable measures by which FCR can be improved remain to be determined.

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Measured nutrient or energy composition of commercial diets fed to juvenile yellowtail kingfish (gkg⁻¹ or MJkg⁻¹ as received basis).

Description	Moisture	Crude Protein	Crude Fat	Ash	Gross Energy
Marine Winter*	63	458	202	90	21.8
Marine NON GMO LAP**	55	454	190	90	22.1
Marine 100% fish oil	62	463	184	92	21.7
Marine 40% fishmeal+ 50 fish oil:50 poultry oil	68	468	201	105	21.1
Marine 75% poultry oil	57	456	209	91	21.9
Marine 50 fish oil:50 poultry oil / 10% blood meal	57	448	195	91	21.8

* Fortified winter diet for yellowtail kingfish **Diet contains no genetically modified ingredients or land animal protein sources

Performance indices (mean±sem) of individual yellowtail kingfish reared at an average water temperature of 16°C for 42 days.

Description	Stock weight (g)	Harvest weight (g)	Feed intake (g/fish)	FCR	Relative weight gain (g kgBWd ⁻¹)	Relative feed intake (g kgBWd ⁻¹)	TGC
Marine Winter*	185 ± 4	306 ± 20	210 ± 3	1.75 ± 0.36	12.02 ± 1.93	21.08 ± 0.74	1.54 ± 0.25
Marine NON GMO LAP**	182 ± 6	280 ± 32	217 ± 11	2.22 ± 0.49	10.22 ± 1.97	22.95 ± 1.42	1.29 ± 0.28
Marine 100% fish oil	185 ± 10	274 ± 16	212 ± 4	2.39 ± 0.38	9.42 ± 1.33	22.56 ± 1.44	1.19 ± 0.17
Marine 40% fishmeal+ 50							
fish oil:50 poultry oil	176 ± 2	280 ± 20	214 ± 3	2.07 ± 0.44	11.05 ± 1.82	22.96 ± 0.81	1.38 ± 0.24
Marine 75% poultry oil	182 ± 7	295 ± 16	209 ± 14	1.85 ± 0.10	11.64 ± 0.43	21.52 ± 1.09	1.48 ± 0.08
Marine 50 fish oil:50							
poultry oil / 10% blood							
meal	174 ± 5	257 ± 11	201 ± 4	2.43 ± 0.26	9.3 ± 0.87	22.63 ± 0.44	1.15 ± 0.11

* Fortified winter diet for yellowtail kingfish **Diet contains no genetically modified ingredients or land animal protein sources

Performance indices (mean±sem) of individual yellowtail kingfish reared at an average water temperature of 23°C for 42 days.

Description	Stock weight (g)	Harvest weight (g)	Feed intake (g/fish)	FCR	Relative weight gain (g kgBWd ⁻¹)	Relative feed intake (g kgBWd ⁻¹)	TGC
Marine Winter*	177 ± 5	489 ± 4	414 ± 7	1.33 ± 0.03	25.27 ± 0.55	33.55 ± 0.41	2.34 ± 0.03
Marine NON GMO LAP**	175 ± 2	428 ± 52	379 ± 46	1.5 ± 0.12	21.97 ± 3.06	32.95 ± 1.93	2.01 ± 0.31
Marine 100% fish oil	179 ± 5	426 ± 38	387 ± 52	1.77 ± 0.42	21.28 ± 2.96	33.9 ± 3.49	1.95 ± 0.28
Marine 40% fishmeal+ 50							
fish oil:50 poultry oil	181 ± 8	460 ± 58	414 ± 27	1.48 ± 0.17	22.94 ± 2.17	34.25 ± 0.63	2.13 ± 0.25
Marine 75% poultry oil	176 ± 5	416 ± 19	405 ± 12	1.69 ± 0.09	21.15 ± 1.00	35.67 ± 0.18	1.93 ± 0.10
Marine 50 fish oil:50							
poultry oil / 10% blood							
meal	181 ± 3	452 ± 33	423 ± 9	1.56 ± 0.16	22.46 ± 1.86	35.21 ± 0.67	2.08 ± 0.19

* Fortified winter diet for yellowtail kingfish **Diet contains no genetically modified ingredients or land animal protein sources
Results of multi factor split-plot ANOVA on selected response variables

Relative Weight gain (g kgBWd⁻¹)

	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp regime	1	1275.68	1275.68	338.20	0.0000*	1.00
B(A): Tank	4	15.0877	3.77194			
C: Diet type	5	40.2180	8.04361	2.38	0.0756	0.63
AC	5	13.37	2.67561	0.79	0.5684	0.22
BC(A)	20	67.6322	3.38161			
S	0	0				
Total (Adjusted)	35	1411.99				
Total	36					

Relative feed intake $(g kgBWd^{-1})$

		Sum of	Mean		Prob	Power
	DF	Squares	Square	F-Ratio	Level	(Alpha=0.05)
A: Temp_regime	1	1267.36	1267.36	407.73	0.0000*	1.00
B(A): Tank	4	12.4333	3.10833			
C: Diet_type	5	10.6566	2.13133	1.49	0.2372	0.41
AC	5	16.78	3.356	2.35	0.0786	0.62
BC(A)	20	28.6	1.43			
S	0	0				
Total (Adjusted)	35	1335.83				
Total	36					

Feed conversion ratio (FCR)

		Sum of	Mean		Prob	Power
	DF	Squares	Square	F-Ratio	Level	(Alpha=0.05)
A: Temp_regime	1	3.56580	3.56580	56.09	0.0017*	0.99
B(A): Tank	4	0.25428	6.357222E-	02		
C: Diet_type	5	0.85769	0.171538	2.34	0.0793	0.62
AC	5	0.57051	0.114102	1.56	0.2177	0.43
BC(A)	20	1.4661	7.330889E-	02		
S	0	0				
Total (Adjusted)	35	6.7144				
Total	36					

Thermal growth coefficient (TGC)

	DF	Sum of Squares	Mean Square	F-Ratio	Prob Level	Power (Alpha=0.05)
A: Temp_regime	1	4.84	4.84	132.10	0.0003*	1.00
B(A): Tank	4	0.14655	0.03663			
C: Diet_type	5	0.52255	0.10451	2.31	0.0820	0.62
AC	5	0.18656	0.03731	0.83	0.5459	0.24
BC(A)	20	0.90337	0.04516			
S	0	0				
Total (Adjusted)	35	6.59905				
Total	36					

Marginal means (\pm pooled sem) of diet type (n=6) or temperature (n=18) on selected response variables.

Description	Relative weight gain (g kgBWd ⁻¹)	Relative feed intake (g kgBWd ⁻¹)	FCR	TGC
Diet type (n=6)				
Marine Winter*	18.65	27.30	1.56	1.94
Marine NON GMO LAP**	16.12	27.95	1.91	1.65
Marine 100% fish oil	15.37	27.93	1.99	1.57
Marine 40% fishmeal+ 50 fish oil:50 poultry oil	17.02	28.62	1.82	1.76
Marine 75% poultry oil	16.40	28.58	1.77	1.71
Marine 50 fish oil:50 poultry oil / 10% blood meal	15.87	28.92	2.01	1.61
Pooled sem	0.75	0.49	0.11	0.11
Temperature (n=18)				
Cold regime	10.62 ^a	22.28 ^a	2.16 ^a	1.34 ^a
Warm regime	22.52 ^b	34.15 ^b	1.53 ^b	2.07 ^b
Pooled sem	0.46	0.42	0.06	0.05

Different superscript letters indicate a significant difference between row means within each factor (Tukey-Kramer; P < 0.05).

* Fortified winter diet for yellowtail kingfish

**Diet contains no genetically modified ingredients or land animal protein sources

Feed composition and properties of three commercial diets fed to *Seriola lalandi*. Data are reproduced from Moran et al. (2009). Data on thermal growth coefficients (TGC) are calculated from growth data presented elsewhere in Moran et al. (2009).

		Manufacturer	
	Salmofood ESF	Skretting NME	Reliance SF
Information supplied by m	anufacturer		
Manufacturing process	extrusion	extrusion	steam pressed
Protein source	fish meal non-ruminant meal plant protein meal	fish meal, plant protein meal poultry meal	fish meal meat & bone meal dried animal blood milk powder
Lipid source	fish oil	fish oil:poultry oil	fish oil
Gross energy MJkg ⁻¹ Ash% Protein% Lipid% Carbohydrate% Moisture% Independent chemical anal Ash% Protein%	23.5 na 43.0 26.0 18.0 10.0 ysis 7.3 49.1 21.4	18.9 9.0 45.0 20.0 18.0 8.0 6.8 41.8	15.6 11.0 45.0 15.0 18.0 10.0 12.7 42.5
Lipid% Free fatty acid%	21.4 3.8	23.2 3.1	6.4
Low temperature period (1 TGC FCR Feed intake %BWday ⁻¹ Protein intake (g kg ⁻¹ day ⁻¹) Energy intake (MJ kg ⁻¹ day	$ \begin{array}{c} 1.09\\ 1.37\\ 0.86\\ 4.2\\ -1 \end{array} $	0.95 1.68 0.97 4.05 183.0	0.68 2.17 1.06 4.5 165.0
High temperature period (TGC FCR Feed intake %BWday ⁻¹ Protein intake (g kg ⁻¹ day ⁻¹) Energy intake (MJ kg ⁻¹ day	$ \begin{array}{c} 17-22C) \\ 1.92 \\ 1.22 \\ 1.41 \\ 6.94 \\ -1) \\ 331.0 \end{array} $	1.91 1.33 1.55 6.48 293.0	1.83 1.46 1.81 7.67 281.0



Seasonal variation in sea surface temperature at Kurnell (NSW), Port Lincoln (SA) and Arno Bay (SA) between August 2005 and August 2007. Data supplied by Bureau of Meteorology (BOM). Data points are fit with sine wave.



Profile of low and high temperature regimes recorded during the experiment.



Plot of theoretical weight gain of yellowtail kingfish stocked at 5g (open circles) or 50g body weight (closed circles). Sea surface temperature profile recorded at Arno Bay (SA) between August 2005 and August 2007 (Bureau of Meteorology). Assumptions: linear relationship between temperature and TGC derived from present study; TGC = 0.1043 x temperature – 0.3286; similar FCR and diets used in present study.

4.12 Digestibility of selected feed ingredients for yellowtail kingfish *Seriola lalandi*

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ABSTRACT

1. INTRODUCTION

Yellowtail kingfish Seriola lalandi is a highly active pelagic marine carnivore belonging to a family which includes the amberjack S. dumerili and yellowtail S. quinqueradiata. Members of the genus share many morphological adaptations with the tunas, including an enhanced metabolic rate (Clark & Seymour, 2006; Pirozzi & Booth, 2009). In Australia, S. lalandi form the basis of a growing sea cage industry approaching 4000 t per annum (Fernandes & Tanner, 2008). Most farming is done in South Australia along side the production of southern blue fin tuna *Thunnus maccoyii*, however S. lalandi is also being trialled in New South Wales and Western Australia. In Australia, they are generally fed extruded dry pellets of variable ingredient and nutrient composition due to the fact that little is known about their nutritional requirements. Nutritional information (e.g. mostly nutrient requirement or fishmeal replacement studies) is available on other Seriola spp., particularly from research conducted in Japan and the Mediterranean. However, direct extrapolation of results to the Australian industry is somewhat difficult given differences between species, availability of feed ingredients, production strategies, environmental conditions and government regulations. In addition, much of the potentially useful nutritional research on species like S quinqueradiata is written and presented in Japanese (see bibliography in Masumoto, 2002). Apart from a couple of rare publications on the digestibility of several protein sources by S. quinqueradiata (Masumoto, Ruchimat, Ito, Hosokawa & Shimeno, 1996; Masumoto, 2002) there is a dearth of published information on the digestibility of feed ingredients for Seriola spp.

Industry and researchers have identified several factors that will limit the expansion and profitability of yellowtail kingfish farming. Key constraints include a lack of knowledge about basic nutritional requirements, optimal diet specifications, digestibility of ingredients, feeding protocols and the effects of environmental parameters such as temperature and salinity on feed intake and nutritional physiology. Collectively, the aforementioned factors represent different but important components of diet development research.

As for most new species, one of the first critical steps in diet development research is the determination of apparent digestibility coefficients (ADC) for a range of potential feed ingredients. Determination of ADCs is important for several reasons. Firstly, it is extremely useful for indicating the nutritional potential of a feed ingredient. Secondly, it permits formulation of research diets on a digestible nutrient basis as it accounts for the potential loss of undigested nutrients and energy from the feed. This allows more rigorous evaluation of feed ingredients and of their prospective inclusion levels, because diets can be compared on a similar digestible protein and energy basis. Thirdly, the uptake of this information by commercial feed companies ensures that the dietary specifications of their proprietary feeds are as consistent as possible. This is particular important where diets are formulated on a least cost basis and component feed ingredients are constantly varied depending on price or availability. Formulating more highly digested feeds based on ADCs also has obvious benefits for the environment by indirectly reducing nutrient outputs from farms (Cho, Hynes, Wood & Yoshida, 1994).

The investigation of potential feed ingredients for aquaculture species remains a priority as the global pressures on fish meal and fish oil continue to escalate (Tacon, 2003). This is especially so

for carnivorous species which have traditionally relied on these two resources to provide their basic dietary needs. This paper describes two experiments that determined the ADCs of dry matter, protein, fat and gross energy of a range of potential feed ingredients for yellowtail kingfish *Seriola lalandi*. The ingredients tested include fish meals, squid and krill meals, rendered animal by-product meals, soybean meal, grain legumes, glutens, extruded wheat and a variety of oils.

2 MATERIALS & METHODS

2.1 Overview

Two separate experiments were conducted to determine the apparent digestibility of selected feed ingredients for yellowtail kingfish. Experiment 1 investigated the digestibility of fishmeal, squid meal, krill meal, meat meal, poultry meal, feather meal, blood meal, soybean meal, dehulled lupin and whole filed peas. Experiment 2 assessed the digestibility of fish oil, poultry oil, canola oil, wheat gluten, maize gluten and extruded wheat. In each experiment either a single (Experiment 1) or a series of reference diets (Experiment 2) were used to investigate the digestibility of test ingredients. Different reference diets were used to ensure that there was little chance of an interaction between specific test ingredients and the ingredients used to compose the reference diet/s. The inclusion content of individual test ingredients were tested at all levels. The digestibility of diets and ingredients was determined using indirect methods with chromic oxide (Cr_2O_3 ; MERCK TechnipurTM, Darmstadt, Germanty) employed as the non-digestible marker. Fish were acclimated to their respective experimental diets for different periods depending on the type of ingredient being studied before faeces were collected. The same fish stock was used in each experiment. A summary of both experiments is presented in Table 1.

2.2 Ingredients and diet preparation

The origin and the measured nutrient and energy content of all test ingredients are presented in Table 2. Prior to inclusion in test feeds all ingredients were ground in a hammer mill fitted with a 1.6mm screen (Raymond Laboratory Mill, Transfield Technologies, Rydalmere, NSW, Australia). The reference diet (or its constituent ingredients), test ingredients and marker were then combined on a dry matter basis and thoroughly mixed (Hobart Mixer; Troy Pty Ltd, Ohio, USA) before the addition of wet ingredients. Each mash was then formed into pellets using a meat grinder fitted with an 8mm diameter die plate (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia). Moist pellets were dried for 5 to 6 h (\approx 35° C) in a simple convection drier until moisture content was < 100 g kg⁻¹ diet. Following preparation, all diets were stored frozen at < -15°C until required. The ingredient composition as well as the measured nutrient and energy content of reference and test diets used in experiment 1 or experiment 2 are presented in Tables 3 and 4, respectively.

2.3 Fish stocks and handling protocols

The yellowtail kingfish used in all experiments were progeny of wild brood-stock held at the NSW DPI Fisheries Port Stephens Fisheries Institute (PSFI). Prior to use in experiments yellowtail kingfish were grown at low densities in large 10 kL holding tanks and fed once or twice daily on a commercial marine fish feed (Ridley Aqua-Feeds Pty. Ltd., Narangba, Qld, Australia).

Groups of yellowtail kingfish were lightly anaesthetised (10-25 mg L^{-1} Aqui-S[®]), individually weighed and transferred from their holding facilities to the digestibility laboratory. The laboratory contained a total of 48 x 200L polyethylene tanks, however replicate groups of fish were only stocked into alternate tanks (24 tanks). This was done to provide a spare, well oxygenated tank in which to recover heavily anaesthetised fish post-stripping. After stocking, 2 replicate tanks were randomly assigned to each dietary treatment. Test diets were fed twice daily (1030h and 1530h) during the acclimation phase and fed 3 times on the day prior to stripping procedures (1030h, 1530h and overnight between 1900-2000h via mechanically operated belt feeders).

Faecal collection was done the morning (0900-1130h) following overnight feeding procedures. This ensured all fish had a minimum of approximately 11h to digest feeds before stripping commenced. In each experiment faecal material was collected by manually stripping fish after they had been rapidly and heavily anaesthetised within their respective experiment tanks (50-60 mgL⁻¹ Aqui-S[®]). Faecal matter was expressed from the distal portion of the digestive tract by applying firm pressure to the abdominal region; running the thumb and forefinger from the pelvic fin region to the vent. Before any material was collected fish were subjected to the same technique in order to expel urinary products and prevent them from contaminating the faecal sample. The area around the vent was wiped clean and faecal matter was expressed into 70mL plastic sample jars. Individual fish were then recovered in the adjacent experiment tank by manually swimming them in well oxygenated water. Daily faecal collections from individual tanks were pooled and kept frozen (< - 15°C) until a sufficient quantity was obtained for chemical analyses. Afterwards, faecal samples were dried for 24h at room temperature in vacuum desiccators (70 mm Hg) using silica as a desiccant. Samples were then finely ground (Waring, model 32 BL 80, New Hartford, Connecticut, USA) and re-dried (as described) prior to chemical analyses.

2.4 Chemical analyses

Proximate chemical analysis of ingredients, diets and faecal material was done by the Queensland Department of Primary Industries & Fisheries (QDPI&F) while analysis of chromium was performed by Ecoteam Environmental Services (University of Sunshine Coast, Qld). Analyses were conducted according to specific in-house methodologies or AOAC (1990). The crude protein content of ingredient, diet or faecal samples was determined by multiplying the measured nitrogen content of each sample by 6.25.

2.5 Calculation of digestibility coefficients

Apparent digestibility coefficients (ADC) of specific nutrients and gross energy for each of the reference and test diets were calculated on a dry matter basis. Prior to calculation of ADC's the analysed nutrient or energy content of test and reference diets was cross checked by deriving the same values from the analysed nutrient or energy contents of individual ingredients as well as ingredient x reference diet combinations. This was done to ensure that spurious ADC's were not determined (Bureau & Hua, 2006). The ADC's for diets were calculated according to equation 1:

ADC (%) = 100 x
$$[1 - (F/D x D_{Cr}/F_{Cr})]$$
,

Eq. 1

where F = % nutrient or gross energy in faeces; D = % nutrient or gross energy in diet; $D_{Cr} = \%$ chromic oxide in diet; $F_{Cr} = \%$ chromic oxide in faeces (Cho, Slinger & Bayley, 1982).

Apparent digestibility coefficients for ingredients were calculated according to equation 2:

$$ADC_{ING} (\%) = [(Nutr_{TD}*AD_{TD}) - (PRD*Nutr_{RD}*AD_{RD})] / [(P_{ING}*Nutr_{ING})], \qquad Eq. 2$$

where ADC_{ING} = apparent digestibility of nutrient or gross energy in the test ingredient; $Nutr_{TD}$ = the nutrient or gross energy concentration in test diet; AD_{TD} = the apparent digestibility of the nutrient or gross energy in the test diet; P_{RD} = proportional amount of reference diet; $Nutr_{RD}$ = the nutrient or gross energy concentration in the reference diet; AD_{RD} is the apparent digestibility of nutrient or gross energy in the reference diet; P_{ING} = proportional amount of test ingredient; $Nutr_{ING}$ is the nutrient or gross energy concentration in the test ingredient (Sugiura, Dong, Rathbone & Hardy, 1998).

2.6 Statistical analyses and figures

The apparent digestibility coefficients of reference diet A determined from experiment 1 or experiment 2 were compared with one-way *ANOVA* setting alpha = 0.05. Data was grouped depending on whether ADC's were calculated using dietary and faecal concentrations of chromium or ash, respectively (i.e. 4 levels: ADC's from experiment 1 based on ash data; ADCs from experiment 2 based on chrome data; ADC's from experiment 2 based on ash data; ADC's from experiment 1 based on recalculated data using functions derived from experiment 2). Percentage data was arcsin-square root transformed prior to *ANOVA* and Tukeys multiple comparison procedure was used to separate treatment means at the 95% confidence interval (Statgraphics Plus Version 4; Manugistics Inc. 1998). Regression analysis and figures were calculated and drawn using GraphPad Prism V4.01 (GraphPad Software, San Diego California USA, www.graphpad.com).

3. **RESULTS**

The measured concentration of chromium in many of the replicate faecal samples collected in experiment 1 was found to be lower than the concentration of chromium in their respective diet samples which resulted in the calculation of negative dry matter digestibility (Table 5). This indicated that either 1) there was a problem with the analysis of chromium in the faecal samples (Saha & Gilbreath, 1991), or 2) chromium oxide was being absorbed or 3) chromium oxide was moving through the digestive tract at a different rate to that of the dry matter. The latter two points violate the general assumptions around the use of inert markers in determination of digestibility (Kotb & Luckey, 1972; Hardy, 1997; Kozloski, de Moraes Flores & Martins, 1998). Due to the extreme variability in the measured concentration of chromium in some of the faecal samples from experiment 1, apparent digestibility coefficients were also calculated using the respective dietary and faecal ash concentrations (muffle furnace determination). The successful use of ash as a digestible marker in the diet of freshwater crayfish has been reported (Jones & De Silva, 1998), however it is not normally used due to the fact that some ash (minerals etc.) is absorbed during the digestive process, violating one of the aforementioned assumptions. However, where the amount of dietary ash is high, as in the case of the test diets used in this experiment, the amount of ash absorbed or returned to the digestive tract is likely to be relatively small. The concentrations of chromium in faecal samples collected in experiment 2 were far higher (e.g. at least 2-4 times the dietary concentration) and less variable than the samples collected in experiment 1. This suggests that analytical error was the most likely cause of the low chromium values in faeces in experiment 1. The estimated digestibility coefficients of diets and ingredients related to experiment 2 were calculated with much greater confidence (Table 6). However, as for experiment 1, ADC's were also calculated using ash concentrations in order to develop a comparative data set.

There was a significant relationship between the faecal ash and faecal chromium concentration of samples collected in experiment 2 ($R^2 = 0.76$, P < 0.0001; Figure 1). When this data was translated into the ratio $\text{Diet}_{Cr}/\text{Faecal}_{Cr}$ (Equation 1) or the same ratio calculated using the ash content of diets and faecal material (i.e. $\text{Diet}_{Ash}/\text{Faecal}_{Ash}$), a moderately strong linear relationship was found ($R^2=0.70$; P < 0.01; Figure 2). This indicated that the use of ash ratios in lieu of chromic oxide ratios to calculate ADC's of diets used in experiment 1 had merit.

The predictable nature of relationships between dietary ADC's determined using chrome or ash from experiment 2 was also investigated. Significant linear relationships existed for dry matter, protein, energy or fat digestibility coefficients, however the relationships were stronger for energy or fat ADC than for dry matter or protein (Figure 2). The linear function for each relationship was;

Dry matter:	$y = 1.408 \pm 0.325 \cdot x - 45.59 \pm 23.57$; R ² =0.51, F _{1,18} =18.79, P<0.001	Eq. 3
Protein:	$y = 1.121 \pm 0.236 \cdot x - 17.42 \pm 20.78$; R ² =0.56, F _{1,18} =22.63, P<0.00	Eq. 4
Energy:	$y = 1.637 \pm 0.187 \cdot x - 65.89 \pm 15.39$; R ² =0.82, F _{1,18} =79.69, P<0.0001	Eq. 5
Fat:	$y = 1.583 \pm 0.089 \cdot x - 58.51 \pm 8.41; R^2 = 0.95, F_{1,18} = 312.1, P < 0.0001$	Eq. 6

Subsequently, these functions were used recalculate dietary and ingredient ADCs for experiment 1. Apparent digestibility coefficients for dry matter, crude protein, gross energy or fat for diets used in experiment 1 or experiment 2 are presented in Tables 5 and 6, respectively. The apparent digestibility coefficients for each test ingredient are presented in Table 7.

The *ANOVA* comparisons of the arc-sin square root transformed ADC's calculated for reference Diet A used in experiment 1 and experiment 2 (Tables 3 and 4) found statistical differences between the method used to estimate dry matter ($F_{3,7}=9.54$, *P*=0.027) or fat digestibility ($F_{3,7}=53.46$, *P*=0.001), but not between methods used to estimate protein ($F_{3,7}=1.31$, *P*=0.386) or gross energy digestibility ($F_{3,7}=3.98$, *P*=0.108). Although *ANOVA* on dry matter was significant, the multiple comparisons procedure could not discriminate between the methods used to estimate the dry matter ADC's of reference diet A at the 95% confidence interval. However, when the interval was relaxed to 90%, the treatments were grouped into two distinct pairs; dry matter ADC's calculated using ash (i.e. Exp.1 = Exp.2) and dry matter ADC's calculated using chromic oxide or estimates of based on recalculation (Exp.1 recalculated = Exp.2 chrome). The method of calculation was also grouped in distinct pairs in tests on fat digestibility, however, in this case estimates of fat digestibility were similar in each experiment (i.e. Exp.1 ash = Exp.1 recalculated; Exp.2 chrome = Exp.2 ash).

4. **DISCUSSION**

The apparent digestibility coefficients of the ingredients tested in experiment 2 have been calculated from original data and are presented with a high degree of confidence. However, data presented for experiment 1 based on the use of chromic oxide marker is not reliable. The determination of ADC's for this experiment were subsequently based on the ratio of dietary to faecal ash content which provided a better estimate of the digestibility of diets than would have otherwise been the case. These 'ash' values were also used to estimate the digestibility of dry matter, protein, energy and fat for each of the ingredients in this experiment, again providing a better estimate of digestibility of these ingredients than was possible using the original analysis of chromium. A further estimate of the digestibility of diets used in experiment 1 was made after recalculating dietary ADC's using functions derived from experiment 2 (Table 7). This was considered a reasonable approach given the similarity in the size of fish, the experimental conditions and the fact that the same reference formula and ingredient base was used in both studies. In addition, statistical comparison of the protein or energy digestibility coefficients determined for reference Diet A using ash, chrome or 'recalculated' values did not differ significantly between experiments. This outcome significantly increased our confidence in estimates of apparent digestibility for ingredients tested in experiment 1. Both the data calculated using ash or derived equations for experiment 1 are presented in Table 7. Overall trends are similar, however in terms of formulating diets we suggest the use of the recalculated values until further digestibility studies are conducted.

The nutrient and energy composition of fish meal originating from Peru or Ecuador was similar (Table 2), however, the dry matter, protein and energy digestibility of fishmeal from Ecuador was superior (Table 7). High quality fish meal is well digested by most fish species and will continue to serve as the benchmark by which other protein sources are judged. The apparent protein digestibility of both fishmeal sources was similar to that reported for S. quinqueradiata fed brown fish meal (88.7%; Masumoto et al., 1996) or a diet containing 80% mackerel mince (87%; Satoh, Hitaka & Kimoto, 2000). Masumoto et al. (1996) also reported the apparent protein digestibility of meat meal (80.3%), soy protein concentrate (87.3%), full fat soybean meal (83.2%) and corn gluten meal (49.7%). Their reported coefficients are slightly higher than for similar ingredients in our study, which may be indicative of the fact that they used modified artemia hatching tanks to collect faces by settlement, sieving and subsequent centrifugation and that their test diets consisted of only the test ingredient of interest, some lipid, binders and feeding stimulants (Masumoto et al., 1996). More recent but unpublished data has been reported on the apparent protein and energy digestibility of fish meal offered to S. quinqueradiata as moist pellets (ADC_{Protein} 89%) or extruded pellets (ADC_{Protein} 84%; ADC_{Energy} 83%), maize gluten meal (ADC_{Protein} 37%; ADC_{Energy} 33%), meat and bone meal (ADC_{Protein} 80%; ADC_{Energy} 82%), meat meal (ADC_{Protein} 97%; ADC_{Energy} 99%), poultryfeather meal (ADC_{Protein} 68%; ADC_{Energy} 70%) and soybean meal (ADC_{Protein} 93%; ADC_{Energy} 62%) (T. Masumoto, unpublished data presented in Masumoto, 2002). Unfortunately there is no description of the methods used to determine the latter coefficients but the cited values for fish meal, poultry-feather meal, meat and bone meal and soybean meal are not dissimilar to the coefficients determined for *S. lalandi* in the present study (Table 7). Protein digestibility of fish and animal meals depends greatly on the processing technique used to render the product (e.g. heating, drum drying, spray drying) and literature values for fish species often vary between 68-92% (Lupatsch, Kissil, Sklan & Pfeffer, 1997). The low digestibility of maize (corn) gluten recorded for *S. lalandi* is mirrored in data presented for *S. quinqueradiata* (Masumoto et al., 1996). In the case of *S. quinqueradiata*, the apparent availability of all amino acids recorded for corn gluten meal were below 51%, so there were no particular amino acids influencing the overall availability of protein. Masumoto et al. (1996) hypothesised the low digestibility might be related to the low pH of the material they tested (pH \approx 3.2).

The dry matter and energy digestibility of whole field peas was low and reflected similar values presented for juvenile mulloway (Chapter 4.2). Whole peas contain significant levels of carbohydrate in the form of fibre which may negatively impact on digestibility (McGoogan & Reigh, 1996). Like mulloway, yellowtail kingfish were also better at digesting dehulled lupin meal than whole field peas. A reduction in the amount of non-digestible CHO present in the diet or ingredient is known to improve dry matter and energy digestibility in many fish species. For example significant improvements in the digestibility of whole vs dehulled legume seeds have been reported in silver perch (Booth, Allan, Frances & Parkinson, 2001). Based on the results of experiment 1, limited levels of solvent extracted soybean meal or dehulled field peas could serve as a useful protein or energy sources in diets for yellowtail kingfish.

The dry matter from fish oil, poultry oil and canola oil was almost completely digested by S lalandi, with the digestibility of fish oil only slightly superior to the other two lipid sources (Table 7). High digestibility of oils and lipids is generally taken for granted, but it is often worth evaluating in order to assess other issues such as the effect of inclusion level (Booth, Allan & Anderson, 2005). The fatty acid composition of these three oils is known to be quite different and although their respective digestibility's are high, the individual impact of each source on the nutrition and health of the fish as well as on the finished lipid composition of whole fish or fillets destined for human consumption will need to be carefully assessed. The fact that there does not appear to be too great a difference between the overall digestibility of the oils we tested indicates that blending of lipids, if desired should not greatly impact on the DE value of formulated feeds when yellowtail kingfish are grown at 22°C. However, lower water temperatures than those used in this study may affect the digestibility of the oils and other high fat ingredients we investigated (Lupatsch et al., 1997). The impact of low temperature on lipid digestibility has been shown to be particularly important in salmonids fed lipid sources containing high levels of saturated fatty acids (high melting point) (Ng, Sigholt & Bell, 2004) as opposed to lipids that are predominated by mono or polyunsaturated fatty acids (Olsen & Ringo, 1998). Given that the bulk of yellowtail kingfish are grown in South Australia where seasonal water temperatures fluctuate between 12-24°C the determination of ADC's for lipids and other nutrients at lower water temperatures is recommended.

The dry matter ADC's of diets containing 0 (Reference diet C), 20, 30 or 40% extruded wheat were significantly different (F3,7=9.86, P=0.026), however, only the extreme values were different from one another (i.e. 0% vs 40% inclusion). The apparent protein, energy and fat ADC's of diets containing 20, 30 or 40% extruded wheat were similar (all P>0.05), but like dry matter ADC, the energy digestibility of the diet containing 40% wheat was numerically lower (Table 6). The similarity in dry matter and energy ADC of diets with up to 30% wheat suggests that the increasing addition of wheat was not overly influencing the apparent digestibility of diets. However, when apparent ADC of ingredients is calculated we see reasonably stable ADC for protein but large variations in dry matter, energy and fat digestibility (Table 7). It was expected that ADC's for dry matter and energy would decline in a systematic manner as the inclusion level of extruded wheat was increased. This type of response has been documented in declining organic matter and gross

energy ADC's of extruded wheat or pregelatinised wheat starch fed to Australian snapper (Booth et al., 2005; Booth, Anderson & Allan, 2006). A systematic response was not completely evident in the dry matter or energy ADC of extruded wheat, which may indicate there was ingredient x reference diet or ingredient x ingredient interactions occurring in this diet series. Because the data indicates that dry matter and energy ADC's of extruded wheat are different at different inclusion levels (i.e unpredictable), the general assumptions about the additive nature of dry matter and energy ADC's related to this ingredient do not hold true (Allan, Rowland, Parkinson, Stone & Jantrarotai, 1999; Glencross, 2009). Lupatsch et al. (1997) also found distinct differences between the measured and predicted CHO digestibility of test diets fed to gilthead sea bream where wheat was the only source of dietary CHO. In terms of ingredient interactions we note that the extruded wheat test diets contained between 1.6-2.4% pre-gelatinised wheat-starch, however these low levels are unlikely to have affected the ADC of extruded wheat.

Historically there has been little research investigating either the digestibility or utilisation of CHO's in diets of *Seriola spp*. One early study investigated the digestibility of dietary CHO (starch vs glucose) by *S. quinqueradiata*. Low values of 56% and 52% were reported, respectively for purified diets containing 10% or 20% alpha-potato starch, but digestibility of CHO from diets containing similar levels of glucose was greater than 90% (Furuichi, Taira & Yone, 1986). Although the digestibility of glucose was high and it entered the bloodstream rapidly, the majority was excreted as urinary products (i.e. glycosuria). The more complex alpha-starch was absorbed slowly and was found to have a minimal impact on blood glucose level which, according to the authors, implied better utilisation (Furuichi et al., 1986).

Many of the same ingredients tested with *S. lalandi* in this trial were also fed to juvenile mulloway in a previous digestibility experiment (Chapter XX). Although digestibility coefficients for mulloway fed the same ingredients at similar inclusion levels were found to be higher (n.b. settlement methods were employed to collect faeces), the overall trend in digestibility of the ingredients was similar. For example the protein and energy digestibility of feather meal by both species was low and that of blood meal was high; the organic matter, dry matter and gross energy digestibility of soybean meal, dehulled lupin meal and whole field peas declined as the NFE content of each ingredient increased. Similarly, the protein from dehulled lupins was better digested by both species than from soybean meal or whole field peas. The comparable nature of these results are encouraging and help support the validity of using endogenous levels of dietary and faecal ash to determine ADC's for *S. lalandi* in experiment 1 (i.e. in the absence of reliable data on faecal markers).

We selected manual stripping over settlement as the most appropriate method of collecting faces from yellowtail kingfish due to the unbound nature of the faeces they produce. Similarly, the use of dissection was ruled out because it would have resulted in the death of a considerable number of large fish which was inconsistent with current animal care and ethics guidelines (Barker, Allan, Rowland & Pickles, 2002). Although stripping methods were employed the recovery of faecal material from large yellowtail kingfish was difficult and problematic. Firstly, the fish had to be rapidly anaesthetised in order to be handled and to prevent the purging of faecal material prior to stripping. Secondly, the recovered amount of faecal material varied from individual to individual and the total amount collected from each tank on a dry matter basis was relatively low (<7.0g). Thirdly, the consistency of faecal material also varied, at times being relatively watery and at other times more dense. Lastly, although recovery from sedation was relatively quick, individual fish required manual swimming or manual ventilation of their gills in highly oxygenated water to ensure mortality did not occur. However, the pre and post-stripping procedures did not appear to affect the re-feeding activity of kingfish which indicated the entire process was probably causing little outward stress to the fish. Similar behavioural responses to re-feeding were found in rainbow trout subjected to repetitive stripping procedures, but repetitive stripping was found to significantly elevate plasma cortisol levels and the expression of certain genes associated with the innate immune response (i.e. tumor necrosis factor-alpha (TNF-alpha) gene) (Stone, Gaylord, Johansen, Overturf, Sealey & Hardy, 2008). Although there were changes in the latter responses, histological examination of the distal section of the digestive tract by the same authors indicated no apparent sign of physical damage due to repeated faecal stripping. Similarly, the authors found no affect of repeated stripping on the digestibility of dietary dry matter, protein, amino acids and gross energy or on ingredient ADCs for dry matter, protein, amino acids and lipid. Some changes were recorded in the ingredient ADC's of dietary lipid, gross energy or minerals (Stone et al., 2008).

The methodology and collection of faecal material from fish remains a difficult issue. Reliable, reproducible methods are only one part of the process because failures or inaccuracies can also occur during chemical analyses which are usually revealed retrospectively and are extremely costly. The calculation of coefficients is also highly dependent on the accuracy of this data and even small variations can lead to large deviations in the calculated result (Bureau & Hua, 2006). In addition, the cost of proximate, gross energy and marker analyses continues to escalate irrespective of the accuracy of results. For example, the chemical analyses alone for each of the experiments described here would run to approximately \$7500. When these costs are coupled to salary and operating costs associated with completing each experiment, the economic risks are even higher. Determination of apparent digestibility coefficients for common and novel feed ingredients remains one of the most critical areas of diet development. For difficult but highly valued species such as yellowtail kingfish, the development of more reliable methods is required. For example, the use of NIR has become part of the nutritional arsenal of most feed manufacturers and use of this technology may prove to be a more cost effective method for obtaining rapid and reliable results from digestibility studies if and when a suitable data base of ingredient x faecal sample composition can be constructed (Picarelli, Greco, Digiovambattista, Ramazzotti, Cedrone, Corazziari & Torsoli, 1995; Aufrère, Graviou, Demarquilly, Perez & Andrieu, 1996; Cozzolino, La Manna & Martins, 2002; Boval, Coates, Lecomte, Decruyenaere & Archimède, 2004; Decruyenaere, Lecomte, Demarquilly, Auffere, Dardenne, Stilmant & Buldgen, 2009).

The digestibility coefficients determined in this study will be useful in formulating new commercial feeds as well as designing specific feeds for use in nutrition experiments with this species. Theses ADC's serve as a useful starting point for constructing a larger data base of ingredient digestibility coefficients for this species.

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Overview of digestibility experiments

	Experiment 1 _{NB808}	Experiment 2 _{NB1008}
Reference diet	formulated	formulated
Test ingredient inclusion (%)	30-50	20-40
Experiment tanks	24	24
Diet replication	2	2
Fish tank ⁻¹	3	3
Mean stock weight (g)	1117±199	1383±268
Stock date	1.9.2008	22.10.2008
Pellet diameter (mm)	8	8
Diet acclimation period (days)	7	7
Temperature (°C)	22±1	22±1
Dissolved oxygen (mg L ⁻¹)	6±1	7±1
Salinity (‰)	29±2	32±1
pH	8±0.5	8±0.5
$^{r}NH_{4}^{+}$ (mg L ⁻¹)	≤1.0	≤0.3

Experiment 1: 1 reference diet; 30% blood meal; 50% all other ingredients Experiment 2: 3 reference diets; 20, 30 or 40% extruded wheat, 20% all other ingredients

Measured nutrient and gross energy composition of ingredients used in experiments (dry matter basis)

	Composition (g kg ⁻¹ or MJ kg ⁻¹)								
Ingredient	Moisture ¹	Organic ² matter	Ash	Crude protein	Fat	Gross energy			
Fishmeal - Peru ^{3,8}	83.0	824.0	176.0	725.0	86.0	20.4			
Fish oil ^{3,5} Poultry oil ⁶ Canola oil ⁷	94.0 - - -			/44.4 - - -	82.0 999 999 999	39.7 39.2 39.7			
Squid meal ^{3,4}	111.0	874.0	126.0	806.9	44.0	21.6			
Krill meal ³	78.0	882.0	118.0	592.5	225.0	24.6			
Meat meal ^{3,6}	45.0	605.0	395.0	500.0	100.0	15.0			
Poultry meal ³	53.0	883.0	117.0	698.8	171.0	23.4			
Feather meal ³	85.0	980.0	20.0	866.9	103.0	24.9			
Blood meal ³	57.0	972.0	28.0	991.9	9.0	24.6			
Soybean meal ³	109.0	938.0	62.0	543.1	28.0	20.2			
Dehulled lupin ³	98.0	974.0	26.0	428.1	73.0	20.8			
Whole field peas ³	88.0	972.0	28.0	231.3	18.0	18.6			
Maize gluten ⁵	63.0	983.0	17.0	633.0	41.0	23.8			
Vital wheat gluten ⁹	69.0	991.0	9.0	821.9	11.0	23.0			
Extruded wheat ³	112.0	973.0	27.0	170.0	46.0	19.0			
Pregell wheat starch ⁵	77.0	998.0	2.0	6.3	9.0	17.3			
RAP Premix ¹²	22.0	461.0	539.0	53.8	72.0	8.2			

¹Moisture value provided to allow calculation of ingredient composition on as fed basis

² Organic matter by difference = (1000 - ash value); all tables

³ Ingredient sourced and provided by Ridley Aquafeed Pty. Ltd., Narangba Qld, Australia

⁴Norway Seafoods; Antarctic krill meal

⁵ Penford Australia Ltd., Lane Cove NSW, Australia ⁶ Australian Meat Holdings (AMH) Pty. Ltd., Dinmore Qld, Australia

⁷ Imported steam dried fish meal with antioxidant, Empresa Pesquera Polar, Ecuador

⁸ Imported steam dried fish meal, high quality, Grupo Sindicato, Pesquero Del Perus, FEMAS, SA

⁹ Manildra Starches, Auburn NSW, Australia

¹⁰ Camalleri Stockfeeds, Maroota NSW, Australia

¹¹ Sunshine Canola Oil (cholesterol free), Steric Trading Pty Ltd, Villawood NSW, Australia

¹² DSM Nutritional Products, Wagga Wagga NSW, Australia

TABLE 3	
Ingredient and measured nutrient composition of diets used in experiment 1 - NB_{808} (g kg ⁻¹ or MJ	kg ⁻¹ dry matter basis)

	Ref -A	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12
Ingredient composition ¹												
Fishmeal - Peru	700	350	350	350	350	350	350	350	490	350	350	350
Vital wheat gluten	80	40	40	40	40	40	40	40	56	40	40	40
Extruded wheat	147	73.5	73.5	73.5	73.5	73.5	73.5	73.5	102.9	73.5	73.5	73.5
Pregell wheat starch	60	30	30	30	30	30	30	30	42	30	30	30
Vit/min premix	3	1.5	1.5	1.5	1.5	1.5	1.5	1.5	2.1	1.5	1.5	1.5
Chromic oxide	10	5	5	5	5	5	5	5	7	5	5	5
Fish meal - Peru	_	500	_	_	_	_	_	_	_	_	_	_
Fish meal - Ecuador	-	_	500	-	-	_	_	-	-	_	_	_
Squid meal	-	-	-	500	-	-	-	-	-	-	-	-
Krill meal	-	_	_	-	500	_	_	-	-	_	_	-
Meat meal	-	_	_	-	_	500	_	-	-	_	_	_
Poultry meal	-	_	-	_	-	_	500	-	-	-	_	-
Feather meal	-	_	_	-	-	_	_	500	-	_	_	_
Blood meal	-	_	-	_	-	-	-	_	300	-	_	_
Sovbean meal (Sol. Ext.)	-	-	_	-	-	_	_	-	-	500	_	_
Dehulled lupin meal	-	_	_	-	-	_	_	-	-	_	500	_
Whole field peas	-	-	-	-	-	-	-	-	-	-	-	500
Nutrient composition												
Organic matter	862	843	847	875	874	752	874	920	893	896	916	917
Ash	138	157	153	125	126	248	126	80	107	104	84	83
Crude protein	605	664	669	704	595	559	641	729	708	567	509	418
Fat	65	74	71	56	133	82	118	88	30	37	64	37
Gross energy	20.0	20.0	20.1	20.5	22.2	17.5	21.6	22.4	21.3	20.0	20.4	19.3

¹ See Table 2 for key to ingredients

TABLE 4		
Ingredient and measured nutrient composition of diets used in	experiment 2 - NB_{1008} (g kg ⁻¹	or MJ kg ⁻¹ dry matter basis)

	Ref-A	Diet 2	Diet 3	Diet 4	Ref-B	Diet 6	Diet 7	Ref-C	Diet 9	Diet 10	Diet 11
Ingredient composition ¹											
Fishmeal - Peru	700	560	560	560	700	560	560	760	608	532	456
Vital wheat gluten	80	64	64	64	-	-	-	200	160	140	120
Extruded wheat	147	117.6	117.6	117.6	147	117.6	117.6	-	-	-	-
Pregell wheat starch	60	48	48	48	140	112	112	27	21.6	18.9	16.2
Vit/min premix	3	2.4	2.4	2.4	3	2.4	2.4	3	2.4	2.1	1.8
Chromic oxide	10	8	8	8	10	8	8	10	8	7	6
Fish oil	-	200	-	-	-	-	-	-	-	-	-
Poultry oil	-	-	200	-	-	-	-	-	-	-	-
Canola oil	-	-	-	200	-	-	-	-	-	-	-
Maize gluten	-	-	-	-	-	200	-	-	-	-	-
Wheat gluten	-	-	-	-	-	-	200	-	-	-	
Extruded wheat	-	-	-	-	-	-	-	-	200	300	400
Nutrient composition											
Organic matter	858	885	891	887	858	891	886	847	875	889	901
Ash	142	115	109	113	142	109	114	153	125	111	99
Crude protein	602	483	484	486	536	556	592	717	602	553	500
Fat	65	253	255	254	63	52	53	67	62	57	56
Gross energy	19.9	23.5	23.9	24.1	19.5	20.1	20.2	20.5	20.2	20.2	20.1

¹ See Table 2 for key to ingredients

		Original chrome basis				Original ash basis			Recalculated basis ¹					
Diet	description	Dry matter	Protein	Energy	Fat	Dry matter	Protein	Energy	Fat	Dry matter	Protein	Energy	Fat	
D1	Reference - A	30.2 35.2	74.3 67.7	58.2 55.2	89.3 90.0	61.2 52.7	85.7 76.4	76.8 67.3	94.0 92.7	75.9 69.8	92.0 83.7	85.3 79.6	96.4 95.5	
D2	Fishmeal - Peru	47.7 35.6	74.0	68.5	92.9	61.6	80.9	76.9	94.8	76.1	87.7 -	85.4	96.9 -	
D3	Fishmeal - Ecuador	59.9 59.8	81.2 81.6	78.2 78.3	94.3 94.3	66.0 65.8	84.1 84.3	81.5 81.5	95.2 95.2	79.3 79.1	90.6 90.8	88.1 88.1	97.1 97.1	
D4	Squid meal	13.0 15.7	48.6	39.2	81.3	57.2	74.7 -	70.1	90.8 -	73.0	82.2	81.3	94.3	
D5	Krill meal	2.6 22.0	50.7	40.8	78.8	65.0	82.3	78.7	92.4	78.5	88.9 -	86.4 -	95.3 -	
D6	Meat meal	-192.0 -90.4	-47.6 -0.9	-48.9 -21.3	64.4 69.8	55.6 46.6	77.6 71.7	77.4 66.0	94.6 91.5	71.9 65.4	84.7 79.5	85.6 78.8	96.7 94.8	
D7	Poultry meal	-57.9 -58.0	0.4	-9.3	46.5	61.5	75.7	73.3	86.9	76.0	83.1	83.2	91.9 -	
D8	Feather meal	-17.7 11.0	2.2	5.8	66.6 -	55.6	63.1	64.4 -	87.4	71.8	71.8	77.9	92.2	
D9	Blood meal	40.3 44.5	73.5	59.6 -	74.1	60.4	82.4	73.2	82.8	75.3	89.0	83.1	89.3	
D10	Soybean meal	-3.7 -10.9	47.8 45.4	23.5 18.0	57.9 49.1	54.6 55.7	77.2 78.2	66.5 67.3	81.6 79.7	71.2 71.9	84.4 85.3	79.2 79.6	88.5 87.3	
D11	Dehulled lupin	-1.3 -18.7	59.2 51.5	23.1 9.2	66.8 33.2	54.6 54.1	81.7 81.2	65.5 64.9	85.1 74.2	71.2 70.8	88.4 87.9	78.6 78.2	90.7 83.8	
D12	Whole field peas	2.9 -39.0	56.8 44.3	21.8 -10.9	34.4 36.2	52.6 45.0	78.9 78.0	61.8 56.1	68.0 74.7	69.7 64.4	85.9 85.1	76.3 72.9	78.9 84.2	

TABLE 5 Apparent digestibility coefficients (%) of test diets determined in experiment $1 - NB_{808}$

Data values are from each replicate tank; missing data values are indicated by a dash. ¹ Dry matter, protein, energy or fat ADC recalculated using equations 3, 4, 5 & 6, respectively.

		Chrome basis			Ash basis					
Diet	description	Dry matter	Protein	Energy	Fat	Dry matter	Protein	Energy	Fat	
D1	Reference - A	69.0	87.0	80.5	86.2	57.7	82.3	73.5	81.1	
		72.3	88.6	82.2	85.1	56.4	82.0	72.0	76.5	
D2	Fish oil	77.7	88.1	87.9	96.5	65.6	81.6	81.3	94.6	
		74.9	88.5	85.7	95.7	65.6	84.2	80.4	94.1	
D3	Poultry oil	74.2	88.2	86.6	100.0	69.3	86.0	84.1	100.0	
		76.5	88.6	85.8	91.5	64.4	82.8	78.5	87.1	
D4	Canola oil	74.4	86.6	85.2	91.4	61.0	79.6	77.4	87.0	
		76.3	89.2	88.1	98.8	69.0	86.0	84.5	98.4	
D5	Deference D	69.0								
D5	Kelerence - B	08.0	-	-	-	-	-	-	-	
D	Malas shatan	/4.6	89.1	82.5	97.2	50.5	/8.8	66.0	94.5	
D6	Maize gluten	64.6	-	-	-	-	-	-	-	
D 7	TT 71 (1)	63.4	80.3	/2.0	92.3	45.8	/0.9	38.5	88.5	
D/	Wheat gluten	72.2	-	-	-	-	-	-	-	
		72.2	88.7	80.5	95.8	53.3	81.0	67.2	92.9	
D8	Reference - C	77.8	89.9	88.1	99.7	64.8	84.0	81.2	99.5	
		78.9	-	-	-	-	-	-	-	
D9	Extruded wheat 20	73.1	89.9	81.7	91.8	53.4	82.4	68.2	85.7	
		68.9	87.0	78.4	91.5	51.6	79.7	66.3	86.7	
D10	Extruded wheat 30	73.4	91.1	81.1	92.5	51.5	83.7	65.6	85.5	
0		73.2	89.8	79.7	89.2	44.5	78.8	58.0	77.6	
D11	Extruded wheat 40	68.3	89.2	76.4	94.3	45.6	81.4	59.4	90.3	
		63.3	87.7	72.1	92.8	46.5	82.1	59.3	89.5	

TABLE 6Apparent digestibility coefficients (%) of test diets determined in experiment 2 – NB1008

Data values are from each replicate tank; missing data values are indicated by a dash.

Apparent digestibility coefficients (%, mean \pm sem) of test ingredients determined in experiment $1-NB_{808}$ and experiment $2-NB_{1008}$

	ADC (%)				
Ingredient	Dry matter	Protein	Energy	Fat	
Experiment 1 NB _{808 (ash ba}	sis)				
Fish meal - Peru	66.2	80.5	81.7	92.4	
Fish meal - Ecuador	74.8±0.2	85.6±0.2	87.7±0.0	90.7±0.0	
Squid meal	57.4	69.5	66.3	92.9	
Krill meal	73.0	82.5	83.8	93.5	
Meat meal	45.3±9.1	68.7±6.6	71.2±13.0	91.8±2.5	
Poultry meal	65.9	68.8	73.7	84.4	
Feather meal	54.3	49.6	57.8	90.3	
Blood meal	68.2	80.6	75.1	na	
Soybean meal	53.3±1.2	71.9±1.0	61.1±0.8	50.7±3.2	
Dehulled lupin meal	51.7±0.5	79.1±0.6	58.4±0.6	56.4±9.6	
Whole field peas	40.6±7.5	71.6±1.7	45.2±5.9	na	
Experiment 1 NB ₈₀₈ (recalcu	ulated basis)				
Fish meal - Peru	79.4	87.3	86.9	94.2	
Fish meal - Ecuador	85.5±0.2	91.6±0.2	90.4±0.0	92.1±0.0	
Squid meal	73.2	77.5	77.9	98.4	
Krill meal	84.2	88.9	89.3	96.6	
Meat meal	71.5±13.5	77.5±5.6	82.0±8.0	94.7±1.6	
Poultry meal	79.2	76.4	83.1	90.3	
Feather meal	70.8	59.6	73.6	96.9	
Blood meal	80.9	86.6	84.2	na	
Soybean meal	70.3±0.8	79.2±0.9	75.5±0.5	69.2±2.0	
Dehulled lupin meal	69.1±0.3	85.5±0.5	74.2±0.4	67.6±6.0	
Whole field peas	61.2±5.4	79.3±1.4	66.6±3.5	na	
Experiment 2 NB _{1008 (chron}	ne basis)				
Fish oil	98.9±7.2	na	95.9±1.4	99.4±0.5	
Poultry oil	94.1±5.5	na	97.3±1.2	99.1±4.6	
Canola oil	94.2±4.7	na	97.3±4.4	98.1±4.2	
Maize gluten	34.7±3.1	50.7	37.4	62.3	
Wheat gluten	75.7±0.0	87.5	73.0	64.2	
Extruded wheat 20	41.8±10.6	89.9	45.4±8.8	89.5±2.4	
Extruded wheat 30	61.5±0.3	96.0±7.0	62.7±2.5	86.4±14.5	
Extruded wheat 40	47.0±6.2	80.4±4.3	53.6±5.7	61.2±4.7	



Relationship between faecal chromium and faecal ash concentration from experiment 2; $y = 1.27\pm0.167 \cdot X + 2.29\pm3.58$, R²=0.76; n=20



FIGURE 2

Relationship between ratio of dietary chromium to faecal chromium and ratio of dietary ash to faecal ash from experiment 2; $y = 1.598 \pm 0.334 \cdot X + 0.0045 \pm 0.097$, R²=0.70.



Relationships between dry matter, protein, energy or fat ADC's determined using chromium or ash concentrations of test diets and faecal material. N.b. outlier in protein relationship is for 20% corn gluten treatment. Refer to text for equations.

4.13 Estimation of protein and energy requirements for yellowtail kingfish Seriola lalandi

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1. INTRODUCTION

Yellowtail kingfish Seriola lalandi is a highly active pelagic marine carnivore belonging to a family which includes the amberjack S. dumerili and Japanese yellowtail S. quinqueradiata. Members of the genus use a carangiform swimming mode, are facultative ram ventilators and share many morphological adaptations with the tunas, including a fusiform body shape and an enhanced metabolic rate (Clark & Seymour, 2006; Pirozzi & Booth, 2009). In Australia, S. lalandi form the basis of a growing sea cage industry approaching 4000 t per annum (Fernandes & Tanner, 2008). Most farming is done in South Australia along side the production of southern blue fin tuna Thunnus maccovii, however S. lalandi is also being trialled in New South Wales and Western Australia. In Australia, they are generally fed extruded dry pellets of variable ingredient and nutrient composition due to the fact that little is known about their nutritional requirements. Nutritional information on other Seriola spp. is available, particularly from studies conducted in Japan and the Mediterranean (e.g. Masumoto, Ruchimat, Ito, Hosokawa & Shimeno, 1996; Ruchimat, Masumoto, Hosokawa, Itoh & Shimeno, 1997; Watanabe, Aoki, Hara, Ikeda, Yamagata, Kiron, Satoh & Watanabe, 1998; Watanabe, Aoki, Yamagata, Kiron, Satoh & Watanabe, 2000a; Watanabe, Hara, Ura, Yada, Kiron, Satoh & Watanabe, 2000b; Watanabe, 2001; Tomas, De La Gandara, Garcia-Gomez, Perez & Jover, 2005; Takakuwa, Fukada, Hosokawa & Masumoto, 2006; Vidal, Garcia, Gomez & Cerdaa, 2008). However, direct extrapolation of results to the Australian industry is somewhat difficult given differences between species, availability of feeds and feed ingredients, production strategies, environmental conditions and government regulations. In addition, much of the potentially useful nutritional research on species such as S quinqueradiata is written and presented in Japanese (see bibliography in Masumoto, 2002).

The factorial or bio-energetic approach to quantifying energy requirements in fish is based on the premise that total energy requirement is the sum of energy needs partitioned for maintenance and growth. Maintenance needs are generally thought to be highly dependant on body size and temperature while the needs for growth are governed by the amount and composition of the added weight gain (Kaushik, 1998; Lupatsch, Kissil, Sklan & Pfeffer, 1998; Lupatsch, Kissil & Sklan, 2001; Bureau, Kaushik & Cho, 2002; Lupatsch, Kissil & Sklan, 2003a; Lupatsch & Kissil, 2005). In its simplest form a factorial model requires the determination of metabolic weight exponents, digestibility of feeds, maintenance requirements and an estimation of the afficiency of protein and energy retention in fast growing fish (Kaushik, 1998). The benefits of the factorial approach are that requirements are not expressed as a percentage of the diet but rather in terms of absolute daily feed requirements per unit of weight and weight gain (Lupatsch *et al.*, 1998).

The conceptual approach to defining energy requirement is generally summarised as follows;

 $DE = M \cdot BW(kg)^b + G \cdot energy gained,$

Where DE = digestible energy requirement in kJ fish⁻¹ d⁻¹; M = coefficient relating metabolic body weight to maintenance energy requirement in kJ BW(kg)^{-b} d⁻¹; G = coefficient predicting the efficiency of energy utilisation for growth and BW = body weight (Lupatsch et al., 2003a). The factorial approach is easily extended to determination of digestible protein or other nutrient requirements and has recently been thoroughly reviewed by Lupatsch (2009). The bioenergetic approach (Kaushik, 1998; Bureau et al., 2002) has been successfully employed to investigate the protein and energy requirements in a variety of aquaculture species including gilthead seabream, white grouper, European seabass, Asian seabass, trout, Atlantic salmon and mulloway (Lupatsch et al., 1998; Lupatsch et al., 2001; Lupatsch et al., 2003a; Lupatsch, Kissil & Sklan, 2003b; Azevedo, van Milgen, Leeson & Bureau, 2005; Lupatsch & Kissil, 2005; Glencross, 2008; Pirozzi, Booth & Allan, 2010a) and has been an important tool in the investigation of energy requirements in terrestrial livestock such as pigs (van Milgen & Noblet, 1999; van Milgen & Noblet, 2003).

This study uses a bio-energetic approach to determine the digestible protein and energy requirements of *S. lalandi* and subsequently predict diet specifications and feeding rates for fast growing fish of different sizes. New research on the routine metabolism of *S. lalandi* has indicated that their optimal temperature for culture is close to 22° C (Pirozzi & Booth, 2009), therefore the various trials presented in this investigation were undertaken at water temperatures between 20- 25° C.

2. MATERIALS & METHODS

2.1 Handling procedures

All *S. lalandi* used in this study were progeny of wild caught brood-stock held at the Industry & Investment NSW Port Stephens Fisheries Institute (PSFI). Prior to use in experiments juveniles were reared at low densities in large 10 kL tanks and fed once or twice daily on a commercial marine finfish feed. Prior to all stocking or harvest procedures kingfish were starved for 24 hours, anaesthetised (5-25 mg L^{-1} Aqui-S), weighed individually and systematically distributed to experiment cages.

2.2 Digestibility of feeds

Two commercially available marine finfish feeds (Com A or Com B) were obtained from different Australian providers and reground through a laboratory scale hammer mill fitted with a 1.5 mm screen (Raymond Laboratory Mill, Transfield Technologies, Rydalmere, NSW, Australia). Chromic oxide (Cr_2O_3 ; BDH GPRTM 99%) and guar gum (Ridley Aquafeed Pty Ltd, Narangba, Australia) were then added to each mash at a concentration of 1g kg⁻¹ and 2 g kg⁻¹ diet, respectively. Each mash was thoroughly dry mixed (Hobart mixer, Troy, Ohio, U.S.A) before being moistened with distilled water and cold pressed into pellets using a simple electric mincing machine fitted with a 10 mm pellet die (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia). Moist pellets were dried on perforated trays in a convection drier at < 40°C until moisture contents were < 100 g kg⁻¹. Pellet strands were broken to < 5 mm in length, sieved of dust and then stored frozen at < -15°C.

Six purpose built 170 L digestibility tanks forming part of a recirculating aquaculture system (for description see Allan, Rowland, Parkinson, Stone & Jantrarotai, 1999) were each stocked with 5 sub-adult *S. lalandi* (mean \pm sd = 361 \pm 107g) and each of the commercial diets was fed to n=3 randomly selected tanks. Fish were acclimated to the marked diets for 10 days prior to the collection of faeces. Fish were fed to apparent satiation once daily (\approx 1500 h) after which each tank was thoroughly cleaned and flushed of uneaten pellets and faecal material. Faecal collection chambers were then packed in ice and faecal material collected overnight by passive settlement. Faeces was collected from each tank once daily (0830 h) for a period of 7 days. Individual tank samples were pooled, dried (<80°C) and stored following similar protocols to those described by Booth, Allan & Anderson (2005). Diet and faecal samples were analysed for dry matter, crude protein (N x 6.25), gross energy, fat, phosphorous (Queensland Health and Nutritional Biochemistry Laboratory, Animal Research Institute, Yeerongpilly, QLD, Australia) and chromium (ECOTEAM, University of the Sunshine Coast, Faculty of Science, Health & Education, Sippy Downs, QLD, Australia).

Water quality parameters were recorded daily (Model 611 Yeo-Kal Electronics, Brookvale, NSW, Australia) with mean \pm sd for temperature (23.7 \pm 0.7°C), dissolved oxygen (6.3 \pm 0.3 mg L⁻¹), pH (7.6 \pm 0.2) and salinity (26.2 \pm 0.8 ‰) remaining within acceptable limits.

Apparent digestibility of the commercial diets was calculated using the equation,

ADC (%) = 100 X [1 - ($F / D \times D_{Cr} / F_{Cr}$)]

Eq. 1

where F = % nutrient or gross energy in faeces; D = % nutrient or gross energy in diet; $D_{Cr} = \%$ chromic oxide in diet; $F_{Cr} = \%$ chromic oxide in faeces (Cho, Slinger & Bayley, 1982). The digestible nutrient or energy content of each diet was calculated by multiplying the dry matter nutrient or energy content of each diet by its associated digestibility coefficient.

2.3 Starvation – metabolic weight exponents of protein and energy loss

Triplicate groups of *S. lalandi* (weight class $\approx 40, 90, 220, 350$ or 500 g) were starved in order to derive the metabolic weight exponents of protein and energy using comparative slaughter techniques. The number of fish stocked into cages varied according to weight class with 20, 10, 4, 4 and 3 individuals stocked into triplicate cages, respectively. Fish were individually weighed in order to minimise weight variance before being placed into one of 15 x 200 L perforated floating cages as described for the utilisation experiment. A representative sample of fish of similar stocking weight to each of the aforementioned weight classes were euthanased and frozen for chemical analysis. Small fish became moribund after 12 days starvation and the experiment was terminated. All fish were euthanased, individually weighed and stored frozen in replicate groups ($\leq 20^{\circ}$ C). The mean \pm sd water temperature during the starvation period was $21.0 \pm 2.2^{\circ}$ C.

The 12 day loss of endogenous protein or gross energy in starved *S. lalandi* was regressed against geometric mean body weight using non-linear analysis techniques to establish the metabolic body weights of protein and energy, respectively. The selected non-linear function used to model both relationships was $y = a \cdot BW(kg)^b$.

2.4 Utilisation – efficiency of protein or energy deposition

The utilisation of feeds examined in the digestibility trial was investigated using comparative slaughter techniques and a restricted feeding regime. An orthogonal arrangement was employed using 2 diets (Com A or Com B), 4 feeding treatments (25% of apparent satiation, 50% of apparent satiation, 75% of apparent satiation or apparent satiation) and 2 weight classes of sub-adult kingfish (120 or 220 g). Each treatment combination was run in duplicate providing a total of 32 experimental units. Six small and 5 large kingfish were stocked into experimental units, respectively, according to their weight class category. These fish were individually selected in order to minimise weight variance at the inception of the trial. Fish were fed their allocated ration once daily between 1100 - 1300 h. Restricted rations were calculated on a daily basis for each *diet x weight* class group after the amount of feed consumed by cages allocated to the satiated treatments were determined.

A representative sample of fish from each weight class was euthanased at stocking and frozen ($\leq 20^{\circ}$ C) for chemical analysis. Similarly, all fish from each cage were euthanased at the conclusion of the experiment (35 days), individually weighed and frozen as a replicate group. Mortality was negligible, however, dead fish were replaced with fin-clipped specimens which were identified at the conclusion of the experiment. These fish were excluded from compositional analyses.

The experiment was carried out under ambient light conditions in a saltwater re-circulation system that consisted of 4 x 10 kL circular fibreglass tanks (3.4 m diameter x 1.2 m depth) housed within a plastic covered shade house at PSFI. Each of these tanks contained 8 cylindrical floating cages (dimensions approximately 0.2 m^3 ; 0.6 m diameter x 0.7 m submerged depth) constructed of 10 mm

perforated plastic mesh. Each cage was fitted with a lid to prevent the escape of fish (1.6 mm plastic mesh). Cages were firmly secured to the outer perimeter of the 10 kL tanks and remained in the same position during the entire experiment. Each 10 kL tank was provided with approximately 60 L min⁻¹ of pre-filtered (sand filter) salt-water pumped from a combination bio-filter sump (5 kL). Effluent water from each experiment tank drained through a 50 mm stand pipe and returned to the sump via gravity flow. Approximately 5-10% of the effluent water was discarded each day and replaced with clean disinfected estuarine water from a reservoir system. Recirculated water was also continuously drawn from the sump and pumped through a commercial foam fractionator. All 10 kL tanks were constantly aerated using a single 250 mm diameter circular air-pad diffuser. The floor of each 10 kL tank was vacuumed at least three times a week to ensure removal of accumulated faecal material and facilitate additional water exchange.

Water quality was monitored daily (Model 611 Yeo-Kal Electronics, Brookvale, NSW, Australia). Total ammonia [NH3 + NH4⁺] was monitored using a rapid test kit procedure (Model 1.08024.0001, E. Merck, Darmstadt, Germany). During the experiment mean \pm sd of temperature, dissolved oxygen, salinity or pH were 23.1 \pm 1.4°C, 6.3 \pm 0.5 mg L⁻¹, 34.4 \pm 0.3 ‰ or 8.0 \pm 0.2 units. Total ammonia [NH3 + NH4+] was always \leq 1.0 mg L⁻¹.

Digestible protein (DP) and digestible energy (DE) intake and crude protein or gross energy deposition data from *S. lalandi* reared in the utilisation trial were referred to the predetermined metabolic weight exponents of protein and energy prior to investigating relationships between intake and deposition. Multiple linear regression analysis was used to ascertain whether there was any difference between the efficiency of protein or energy utilisation of commercial diets (i.e. comparison of regression lines) and to determine the partial efficiencies of protein and fat deposition with respect to DE intake (Statgraphics Plus for Windows, Version 4.1, Manugistics Inc., Maryland U.S.A; <u>www.statgraphics.com</u>).

2.5 Growth model and composition of whole fish

A model of the potential growth rate of *S. lalandi* was established by recording systematic increases in the body weight and fork length of fish used in experiments similar to those presented here as well as in populations of fish reared in large holding tanks located at PSFI. The total data set is an amalgamation of several years research on different cohorts of kingfish fed a range of different feed types and feed composition (e.g. weaning, nursery, sub-adult or adult stages). In order to ensure that the predictive growth model was representative of potential growth rates in farmed kingfish, any questionable or unusual growth data was not included in our model. Growth rates are based on fish reared between 20-25°C. The same principles were applied to model potential feed intake of fish fed to apparent satiety. Similarly, rapidly growing kingfish of various body weights were selected from within the general population of stocks at PSFI and from specific growth experiments to establish relationships between whole body weight and the nutrient or energy content of whole fish. No starved or restrictively fed kingfish were used in either growth or composition models.

2.6 Carcass preparation

Whole groups of frozen fish were thawed to $\leq 2^{\circ}$ C and where necessary cut into blocks. Fish were then passed multiple times through a meat mincer (Barnco Australia Pty Ltd, Leichhardt, NSW, Australia) fitted with a 3 mm die until each sample group was thoroughly homogenised. A sub sample of fish paste was then refrozen and dispatched under dry ice for analysis of dry matter, crude protein (N x 6.25), gross energy, ash and fat (Queensland Health and Nutritional Biochemistry Laboratory, Animal Research Institute, Yeerongpilly, QLD, Australia).

2.7 Curve fitting

Linear or non-linear models were iteratively fit to starvation, growth, feed intake and utilisation data using GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego California USA, www.graphpad.com).

3. **RESULTS**

3.1 Digestibility of feeds

The gross nutrient and digestible composition of the commercial feeds are presented in Table 1. All fish were lost from one replicate tank assigned to diet Com B due to equipment failure and before sufficient faeces could be collected for analysis. As such, individual replicate values for this diet are given in Table 1. The average of these 2 values was used to calculate the digestible nutrient and energy intake of kingfish assigned to this diet in the utilisation trial.

3.2 Starvation – metabolic weight exponents of protein and energy

The daily loss of endogenous protein, energy or lipid in *S. lalandi* held at a water temperature of 21.0 ± 2.2 °C is presented in Table 2. Data and regression curves for protein and energy loss are presented in Fig. 1a and Fig. 1b, respectively. The following relationships were derived from the raw data;

Protein loss (g fish	$^{1} d^{-1}$) = -1.13±0.2	$3 \cdot BW(kg)^{0.68\pm0.17};$	$R^2 = 0.66$	Eq. 2
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Energy loss (kJ fish⁻¹ d⁻¹) = $-101.00 \pm 13.00 \cdot BW(kg)^{0.86 \pm 0.12}$; R²=0.88 Eq. 3

Exponents for protein and energy indicate that the rate of loss in these body stores decreases as fish weight increases. Much conjecture surrounds the application and use of exponents describing allometric relationships. Therefore, the null hypothesis that the derived exponent for the metabolic body weight of protein was equal to 0.7 or that the exponent for energy was equal to 0.8 was investigated using a comparison of fits test on the same data sets with α set at 0.05. In both cases an F-test indicated that the hypothetical values of 0.7 (F_{1,13} = 0.01026) or 0.8 (F_{1,13} = 0.2252) should not be rejected in favour of our unconstrained exponent values. Constraining each exponent also reduced the standard error in each of the related coefficients (Eq. 2a and 3a). In terms of energy loss, the derived coefficient is representative of fasting heat production (HEf) (NRC, 1993; Bureau et al., 2002) at the aforementioned temperature, however as *S. lalandi* are a highly active species this value also includes a considerable amount of voluntary swimming activity.

	Protein loss (g fish ⁻	d^{-1}) = -1.15±0.10•BW(kg) ^{0.70} ; R ₂	=0.66 Eq. 2a
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	Energy loss (kJ fish ⁻	$^{1} d^{-1}$ = -95.45±4	4.88•BW(kg) ^{0.9}	⁸⁰ ; R ₂ =0.88	Eq. (3a
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3.3 Growth, feed intake and composition of whole fish

A non-linear function in the form of $y = a \cdot BW(g)^b$ adequately described the relationship between daily growth rate and geometric mean body weight for *S. lalandi* reared at temperatures between 20-25°C (Fig. 3a, n=124 data points).

Growth rate (g fish⁻¹ d⁻¹) =
$$0.268 \pm 0.033 \cdot BW(g)^{0.52 \pm 0.02}$$
; R²=0.95 Eq. 4

The same non linear function was used to describe the relationship between fork length and body weight (Fig. 3b, n=586 data points).

Fork length (mm) =
$$45.09 \pm 0.59 \cdot BW(g)^{0.31 \pm 0.002}$$
; R²=0.98 Eq. 5

Relative feed in *S. lalandi* declined as fish weight increased and data from various experiments was described by the power function (Fig. 4, n=174 data points).

Relative feed intake % BWd⁻¹ = $21.14 \pm 1.02 \cdot BW(g)^{-0.3805 \pm 0.012}$: R²=0.83 Eq. 6

The chemical composition of whole fish changed as fish weight increased. Moisture content decreased and dry matter content increased in a linear fashion and the slope of either relationship was significantly non-zero ($F_{1,27} = 77$, *P*<0.0001) (Fig. 5a and 5b).

Dry matter (%) = $0.0098\pm0.0011 \cdot BW(g) + 25.10\pm0.51$; R₂=0.74 Eq. 8

A regression of protein content on body weight indicated the resultant slope was not significantly different from zero ($F_{1,27} = 4.146$, *P*=0.0517; Fig. 5c). This supported the premise that whole body protein content was relatively constant over the range of body weights we investigated. Therefore the mean of n=29 values was used to predict the whole body protein content of fish.

Protein content (%) = 18.82±0.17•BW(g)

Whole body amino acid composition was determined on 4 groups of homogenised fish ranging from approximately 60 to 600g. The results indicated there was no major difference in the relative content of IAA or NIAA when data was expressed as a percentage of whole body protein content (Table 3).

The relationships between whole body gross energy, whole body fat or whole body ash content and increasing body weight varied in a non-linear fashion and were described best by the function $y = a \cdot BW(g)^{b}$ (Fig. 5d, 5e and 5f).

Energy content (kJ g ⁻¹) = $2.79 \pm 0.27 \cdot BW(g)^{0.157 \pm 0.017}$; R ² =0.78	Eq. 10
Fat content (%) = $0.35 \pm 0.09 \cdot BW(g)^{0.506 \pm 0.0421}$; R ² =0.88	Eq. 11
Ash content (%) = $5.09 \pm 0.33 \cdot BW(g)^{-0.079 \pm 0.013}$; R ² =0.59	Eq. 12

3.4 Utilisation – efficiency of protein or energy deposition

Data on average weight gain, feed intake, feed conversion ratio (FCR) and digestible nutrient or energy intake as well as gross nutrient or energy deposition is presented in Table 4. Regardless of diet type, all kingfish that were fed to apparent satiation grew rapidly, more than doubling their stocking weight after 35 days. Average FCR ranged from 1.39 (best) to 2.19 (worst) and with the exception of large fish reared on COM B, there appeared to be a trend towards improved FCR in fish fed at 75% of apparent satiation. Raw data was subsequently referred to the aforementioned fixed metabolic weight exponents of 0.7 (protein) or 0.8 (energy) prior to further statistical or graphical analyses to remove issues associated with fish size.

Multiple regression analysis indicated that linear models to describe the relationships between protein gain, DP intake and diet type or energy gain, DE intake and diet type were both highly significant (P<0.0001). Follow up tests to determine the statistical significance of the terms in each model (i.e. conditional sums of squares) indicated that while both DP intake and DE intake were highly significant (P<0.0001), there was no significant difference between the slopes or intercepts of either model (P>0.1). This indicated a similar efficiency (i.e. regression coefficient) of protein or energy utilisation for each diet. Subsequently data was pooled across diet to establish the efficiency of DP and DE utilisation above maintenance.

Eq. 9

Protein gain = 0.41±0.03•DP intake – 0.14±0.17; R ² =0.90	Eq. 13
Totem gam – 0.41±0.05°D1 mtake – 0.14±0.17, K –0.90	Eq. 15

Energy gain = 0.55±0.03•DE intake – 29.66±8.36; R²=0.92 Eq. 14

The reciprocal value of the regression coefficient in each model is indicative of the unit cost to deposit protein or energy in fast growing kingfish above maintenance using practical commercial feeds. The reciprocal value is 2.44 for protein and 1.82 for energy.

Incorporating data for larger fish (GMBW > 180 g) from the starvation trial permitted the fitting of a linear model which covered the range of DP and DE intake values above and below the maintenance feeding ration. The linear regressions for protein and energy utilisation under this scenario are;

Protein gain = $0.51 \pm 0.02 \cdot DP$ intake - 0.89 ± 0.14 ; R ² =0.94	Eq. 15
Energy gain = 0.65±0.02•DE intake – 60.88±6.25; R ² =0.95	Eq. 16

Although a linear fit to these data sets was adequate, a curvilinear model of the form $y=a \cdot (1 - exp^{(-b \cdot (x-c))})$ described the data somewhat better. The regression parameters of each relationship are presented below while the curvilinear response is presented graphically in Fig 2.

Protein gain = $a \cdot (1 - exp^{(-b \cdot (DP \text{ intake-c}))})$ where;

a=6.216±0.793		
b=0.114±0.019		
c=1.702+0.114	$R^2 = 0.97$	Eq. 17

Energy gain = $a \cdot (1 - exp^{(-b \cdot (DE intake - c))})$ where;

a=416.1±80.36		
b=0.0019±0.0005		
c=87.44±5.003	$R^2 = 0.97$	Eq. 18

Maintenance protein and energy requirements were estimated from Eq. 17 and Eq. 18 at the point of zero protein or energy deposition, respectively (i.e. x-intercept; parameter c),

Maintenance protein requirement = 1.702 g DP BW(kg) ^{-0.70} d ⁻¹	Eq. 17a
Maintenance energy requirement = 87.44 kJ DE BW(kg) ^{-0.80} d ⁻¹	Eq. 18b

The voluntary intake of DP and DE that supported maximum protein or energy deposition in this study was predicted from the asymptote of Eq. 17 or Eq. 18, respectively (i.e. parameter a).

DP requirement for maximum prot	tein gain = 6.22	g DP BW(kg) [,] d	- Eq. 17b
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DE requirement for maximum energy gain = 416 kJ DE BW(kg)^{-0.80} d⁻¹ Eq. 18b

Energy equivalents for protein and energy were calculated from the proximate compositional data set using the following multiple linear regression (n=32);

Energy gain (kJ) = a•protein gain + b•lipid gain

Parameter estimates for the energy equivalents of protein and lipid in *S. lalandi* from this study were found to be $a = 23.07 \pm 1.11$ kJ g⁻¹ and $b = 35.51 \pm 1.94$ kJ g⁻¹, respectively.

Eq. 19

The partial energetic efficiencies of protein and lipid deposition were estimated by multiple regression of DE intake on the energy equivalents of protein and lipid deposition according to the following model (Lupatsch et al., 2003a; Azevedo et al., 2005);

DE intake = $HE_m + 1/k_P \cdot PD + 1/K_L LD$,

Eq. 20

where $DE = digestible energy intake in kJ BW(kg)^{-0.8} d^{-1}$, $HE_m = the maintenance energy requirement in kJ BW(kg)^{-0.8} d^{-1}$, $PD = protein deposition in kJ BW(kg)^{-0.8} d^{-1}$; $LD = lipid deposition in kJ BW(kg)^{-0.8} d^{-1}$ and k_P and k_F are coefficients that estimate the energetic efficiencies of protein and lipid deposition, respectively. The data set for this analysis included fish from the utilisation study as well as starved fish > 180g GMBW. Preliminary analysis indicated that one observation from the starved group had a larger than normal studentized residual and this outlier was removed from the regression analysis.

Results of the multiple regression indicated there was a significant relationship between the independent variables and DE intake ($F_{2,37}$ =299.54, *P*<0.0001, R_2 =0.94). The parameter estimates ± SE of the fitted model were; constant or intercept =101.87±12.69, 1/K_P = 1.64±0.45, 1/K_L = 1.21±0.38. K_P and K_L, the energetic efficiency with which protein or lipid were deposited were subsequently determined as 0.61 and 0.83, respectively.

3.5 Diet specifications and predicted feeding rates

Maximum potential growth rate, maintenance requirements and cost of production estimates were used to predict the DP and DE requirements of rapidly growing *S. lalandi* up to 2kg in body weight. Maximum growth rate was determined by adding the appropriate standard error to both the coefficient and exponent of Eq. 4, respectively. Iterative diet specifications were based on feeds formulated to contain 12, 15 or 18 MJ DE kg⁻¹. For clarity, the relevant equations used to calculate tabulated values are presented in the footnotes to each table (Table 4 and 5).

Piecewise regression analysis (Hintze, 2006) was used to fit a linear-linear model to the relationship between theoretical DP:DE ratio for growing *S. lalandi* and increasing body weight (Table 4) in order to identify key growth stages (Pirozzi et al., 2010a) and recommend appropriate diet specifications for practical commercial diets. The piecewise model was fit to a data set based on 50g increments in fish weight. The three linear sections estimated from the analysis were,

DP:DE ratio = -0.0717•BW(g) + 47.755 where BW ≤ break 1	Eq. 21
DP:DE ratio = -0.0085•BW(g) + 34.840 where break $1 \le BW \le break 2$	Eq. 22
DP:DE ratio = -0.0020•BW(g) + 28.618 where BW > break 2	Eq. 23

Breakpoint 1 and breakpoint 2 were determined at body weights of 204.5g and 963.1 g, respectively (Fig. 6).

4. **DISCUSSION**

4.1 Digestibility of commercial feeds

Although there was no difference between the protein or energy utilisation of the commercial feeds used in our study, the digestibility of the diets was lower than we expected. This may be due to the quality of the ingredients used in each diet or possibly to the elevated water temperature and feeding rate used in the digestibility study. Watanabe et al. (1998) studied feed intake of *S. quinqueradiata* under winter conditions and speculated that gastric evacuation rate would be faster at elevated water temperatures, subsequently increasing feed intake but reducing digestibility. Alternatively, there is

recent evidence showing that the restoration rate of trypsin in the pyloric cecae – pancreatic tissues of *S. quinqueradiata* can take up to 48h, suggesting that if fish are fed on a daily basis the efficiency of protein digestion may be reduced compared with less frequent feeding (Murashita, Kubota, Kofuji, Hosokawa & Masumoto, 2005). Low water temperatures coupled with low dietary protein level has also been shown to affect the content and activity of proteases in *S. dumerili*, resulting in lower protein digestibility of feeds (Kofuji, Akimoto, Hosokawa & Masumoto, 2005).

The apparent protein digestibility of the two commercial feeds used in our study (i.e. 59-72%) brackets that cited for *S. quinqueradiata* fed a series of test diets containing 380 g crude protein kg⁻¹ diet (Kofuji et al., 2006). In that study coefficients were determined by dissection and were considerably lower (60-73%) than protein ADC's recorded for *S. quinqueradiata* fed soybean meal diets where faeces was collected by sedimentation (84-86%) (Tomas et al., 2005). For *S. dumerili* subjected to stripping techniques, protein ADC of diets containing different crude protein to lipid ratios was relatively stable (\approx 80%), however the energy ADC of the same diets ranged from about 68-81% (Takakuwa et al., 2006). Dietary protein (as casein) and glucose (>91%) were readily digested by *S. quinqueradiata*, but only about 52-56% of the starch (α -potato) in the same diets was digested (Furuichi, Taira & Yone, 1986). An explanation for the low digestibility of the propriety aqua-feeds used in our study will remain elusive until robust data on the digestibility of feed ingredients is determined for *S. lalandi*.

Many bio-energetic studies rely on the use of test diets containing high levels of good quality fishmeal and fish oil to determine both the apparent digestibility of protein and energy as well as determination of utilisation efficiency coefficients. This approach is usually advocated based on the fact that fishmeal and fish oil are the most adequate source of essential amino and fatty acids, respectively, for fish. Experimental feeds such as these are likely to be highly digestible and well utilised, however, feeds of commercial origin are far more variable due to the fact they generally contain a variety of protein and energy sources of varying quality and inclusion content. In addition, as the current aim of most feed manufacturers is to reduce the level of fishmeal and fish oil in their formulations to reduce costs, the likelihood that S. lalandi will be cultured on diets containing high levels of fishmeal and fish oil in future years is low. For these reasons we selected and fed two commercial aquafeeds to develop a bio-energetic model for S. lalandi. In taking this approach it is important to ensure that the diets themselves were not nutritionally deficient. Evidence that the diets we selected provided adequate amounts of DP and DE is supported by the fact that although the apparent digestibility of the diets was different, the subsequent maintenance requirements and utilisation coefficients of DP and DE for each diet were the same. Similarity in the utilisation coefficients of DP and DE by gilthead seabream fed diets with varying DP:DE content has also been reported (Lupatsch et al., 2003b). In addition, although diet COM B contained a higher level of DP and DE than diet COM A, both diets contained a very similar DP:DE ratio $(27.1 - 29.3g \text{ DP MJ DE}^{-1})$ ¹). Thus the slight improvement in growth and protein deposition of S. lalandi fed to apparent satiation on diet COM B likely reflects the fact that this diet contained slightly more DP and DE in absolute terms (i.e. more DP and DE per unit of intake) than diet COM A and that S. lalandi consumed slightly more of it. This evidence also supports the fact that depending on temperature, feed intake in S. lalandi may be governed by mechanisms associated more strongly with stomach fullness rather than the DE or DP density of feeds, at least under similar feeding regimes to that used in our study. This hypothesis needs further clarification.

4.2 Weight exponents

A starvation trial was used to determine the metabolic weight exponents of energy and protein, an indirect approach that is well documented in numerous publications on fish (Hepher, 1988; Lupatsch et al., 1998; Lupatsch et al., 2001; Lupatsch et al., 2003a; Lupatsch & Kissil, 2005; Glencross, 2008; Lupatsch, 2009; Dumas, France & Bureau, 2010). Weight loss in starved fish was rapid and described by a power function of the form; $y = 3.415\pm0.4622 \cdot x^{-0.3720\pm0.0489}$ where y = loss in g kgBW^{-0.8} d⁻¹ and x = geometric mean body weight in kg (extrapolated from Table 2).

Exponential modelling of raw data estimated the weight exponents of protein and energy to be 0.68 and 0.86, respectively, however these exponents were not found to be statistically different to 0.7 or 0.8, values that improved the error associated with the coefficients estimating fasting heat production (HEf) and protein loss. These exponent values are now commonly used to standardise relationships between metabolism and fish weight for species such as barramundi (Glencross, 2008), gilthead seabream (Lupatsch et al., 1998), European seabass, white grouper (Lupatsch et al., 2003a) and mulloway (Pirozzi et al., 2010a) and are useful for comparing data between different species.

4.3 Growth, feed intake and composition of whole fish

Well fed S. lalandi grew rapidly when reared at temperatures above 20°C whether they were held in small cages or larger tanks. Weight gains of greater than 12 g fish⁻¹ d⁻¹ were measured in fish weighing more than 1 kg in body weight and weight gain of more than 17g fish d⁻¹ should be expected in fish approaching 2 kg body weight (Table 4). These rapid gains were due in part to the fact that S. lalandi in the present study were reared at close to their optimal water temperature (i.e. 22°C; Pirozzi & Booth, 2009). Although weight gain was highest in fish fed to apparent satiation, FCR was slightly better (lower) in fish fed 75% of a satiated ration, regardless of diet. This phenomenon is common in feeding studies where feed intake is slightly restricted (Brett, 1979) and suggests that rations just below 100% of apparent satiation may promote more economical use of feeds by S. lalandi. The FCR for fish recorded in our study was similar to that recorded for New Zealand S. lalandi fed a variety of commercial feeds at temperatures above 17°C (Moran, Pether & Lee, 2009). In contrast the SGR of fish raised in the latter half of their feeding trial (ca. 1.2% BWd⁻ ¹) was at least half that calculated for fish reared in our feeding study (range 2.0-3.0% BWd⁻¹). This difference is probably due to the difference in the size of experimental animals between studies and the variable nature of the ambient water temperatures they used (i.e. 17-22°C) rather than diets per se. Based on extrapolation of our growth model (Eq. 4) the SGR of S. lalandi reared at water temperatures > 20°C could be described as SGR (% BWd^{-1}) = 1.22• $BW^{-0.458}$ where BW equals the geometric mean body weight in kg. Predictions of SGR for 1 kg fish using this model agree well with the measured values presented by Moran et al. (2009) and are in line with SGR published for different sized S. quinqueradiata (Talbot, Garcia-Gomez, De La Gandara & Muraccioli, 1999).

An important concept in using the factorial approach to predict suitable diet specifications for fish is an understanding of potential feed intake of the species under study. We modelled feed intake in fish of various sizes based on data collected from several experiments (Eq. 6). In most cases fish were offered feed twice daily to apparent satiation and experimental or husbandry temperatures were $> 20^{\circ}$ C. Feeds used in different experiments were typical of those used in the present study with between 45-52% crude protein and 17-22 MJ GE kg⁻¹. It is clear from our data that relative feed intake in S. lalandi declines systematically with increasing body weight. Small rapidly growing fish were able to consume approximately 10% BW d⁻¹ declining to approximately 1.5% BW d⁻¹ in fish close to 1kg BW (Fig. 4). According to the factorial model (Tables 4 and 5), absolute and relative feed requirements for fish of different sizes change depending on the level of dietary DE selected; feed intake of diets with a low energy density increases while feed intake of diets with higher energy density decreases. Therefore it is extremely important to ensure that when formulating feeds containing lower levels of DE or DP that fish are able to physically consume enough of the feed to satisfy their total daily protein and energy requirements. If this is not the case growth will not be optimised due to a greater relative proportion of ingested energy and protein being consumed by maintenance demands.

Dry matter, gross energy, fat and ash content of whole *S. lalandi* varied with fish size, however protein content of whole fish was relatively stable. Not surprisingly, the amino acid composition of growing fish was also similar when expressed as a percentage of crude protein. These compositional relationships are common in other species examined by factorial modelling (Lupatsch et al., 1998; Lupatsch et al., 2001; Lupatsch et al., 2003a; Glencross, 2006; Pirozzi et al., 2010a). Energy density, as a result of the increasing proportion of whole carcass fat and decreasing moisture

content, increased dramatically over the growing range of fish we studied, especially in the first 300g (Fig 5d). This dramatic increase in the energy density of the added weight gain in *S. lalandi* is an important factor governing the increasing energy requirement of this species as it grows. There has been recent speculation that fish species which have a higher relative energy content per unit of weight might require higher levels of dietary energy per unit of weight gain than leaner fish (Lupatsch, 2009). In our case, the energy content per unit of whole live weight was similar to that of white grouper, Asian seabass and tilapia, but lower than gilthead seabream (Lupatsch, 2009). However, despite similarities in energy density the energy demands of *S. lalandi* were found to be much higher than any of these species (see below). This departure, apart from dietary influences, is likely explained by the dramatically higher growth rate of *S. lalandi* compared to the other species, as energy requirements are dependant on the absolute amount of added gain as well as its composition. Of the comparative species mentioned, white grouper have the fastest growth rate, however this rate represents only about 50-65% the daily gain of similar sized *S. lalandi*.

4.4 Fasting heat production HEf

Heat production (HE), or loss of metabolisable energy (ME) in fish and animals is normally partitioned into three main components; heat of nutrient metabolism (HiE), physical activity (HjE) and basal metabolism (HeE). HiE has been coined "an unavoidable tax on energy consumption" and occurs whether fish are in a fed or fasting state (Hepher, 1988). Therefore our comparative slaughter methodology estimates heat production in starved, free swimming S. lalandi. As such our estimates are a collective measure of energy demands more commonly known as HEf in starved animals and maintenance energy (HEm) in fed animals. In animal production, measurement of HEf is generally considered analogous to HEm (NRC, 1993), although from a physiologically standpoint this is incorrect (van Milgen & Noblet, 2003). In addition, our results are based on the determination of DE intake and not on ME intake. Thus, our data does not allow estimation of energy losses due to gill and urinary excretions (ZE + UE) (Cho et al., 1982; NRC, 1993; Bureau et al., 2002). These losses have been estimated to be between 2.3-3.5% of DE intake in very small yellowtail (Ruchimat et al. cited in Watanabe, Ura, Yada, Kiron, Satoh & Watanabe, 2000c) and 2.5-4.0 kJ kgBW⁻¹ d⁻¹ in marine fish such as turbot and gilthead seabream. Marine fish species appear to retain a much lower proportion of the digestible protein fed to them than salmonid fish species and thus have much higher ZE + UE values (Kaushik 1998), however, non faecal energy losses in fish such as ZE + UE are considered to be relatively small in terms of overall energy intake (Bureau et al., 2002).

Recent research on the routine metabolic rate (RMR) of *S. lalandi* has provided important comparative data on this species, particularly with regard to estimates of HEf predicted from the present study (i.e. HEf = 95.5 kJ kgBW^{-0.8} d⁻¹; Eq. 3a). In the study of Pirozzi & Booth (2009), the HEf of 200g juvenile *S. lalandi* was determined at several temperatures ranging from 10 to 32°C by measuring the uptake of oxygen from water by small schools of free swimming fish that had been deprived of feed for 48 hours. Thus, the metabolic rate of these fish was not affected by long periods of starvation nor, presumably an elevated HiE, but it did include heat production associated with voluntary activity HjE. Based on their work they were able to establish a relationship between temperature and routine metabolic rate (RMR) and convert this relationship to energy equivalents using a standard oxy-energetic coefficient of 13.59 kJ g⁻¹ O₂. Thus, the daily energetic cost of postabor provide the data of the set of

Daily energy requirement (kJ kgBW^{-0.8} day⁻¹) = 4.041•T-13.141 Eq. 24

where T= water temperature. Use of this equality subsequently allowed prediction of the HEf of fish reared at the lower and upper end of the temperature range recorded in our utilisation trial (i.e. $23.1\pm1.4^{\circ}$ C). The calculated values according to the equality of Pirozzi & Booth (2009) ranged between 80.2 and 85.9 kJ kgBW^{-0.8} d⁻¹. These derived values are slightly lower than the HEf determined for starved, free swimming *S. lalandi* in the present study which likely reflect differences in experimental approach (respirometer vs carcass slaughter) and the duration of feed deprivation prior to measurement or slaughter (48h vs 12 days). Regardless of these differences the
HEf values from these two studies are quite similar. Based on results of Eq. 3a the rate of energy loss (HEf) in *S. lalandi* is at least double that predicted for mulloway (Pirozzi & Booth, 2009) and gilthead sea bream, nearly 3 times that predicted for European sea bass and almost four times that predicted for white grouper raised at temperatures between 17-27°C (Lupatsch et al., 2003a; Lupatsch & Kissil, 2005).

Others have also recently investigated the metabolic demands of *S. lalandi* (Clark & Seymour, 2006). These authors estimated the standard aerobic metabolic rate (SMR) of fasted (30h), 2 kg *S. lalandi* in a swim tunnel respirometer by extrapolating the oxygen consumption rates of swimming fish to zero swimming velocity at two temperatures (20 or 25°C). In either case the SMR at zero velocity was estimated to be 1.55 and 3.31 mg kg⁻¹ min⁻¹, at 20°C or 25°C, respectively. This equated to 30.3 and 64.8 kJ kgBW⁻¹ d⁻¹ using the aforementioned conversion coefficient. Standardising these values to a weight exponent of b=0.8 resulted in estimates of 34.8 and 74.5 kJ kgBW^{-0.8} d⁻¹ for each of the respective temperatures. As these values were extrapolated from zero swimming velocity they reportedly represent near basal metabolism at each temperature (HeE). The fact that the higher of these estimates is somewhat similar to the values we report for free swimming fish may indicate that the individual fish used in their study were somewhat stressed by confinement in a tunnel respirometer.

4.5 Maintenance requirements HEm

In this study both HEf and HEm could be estimated from the linear (Eq. 16) and non-linear (Eq. 18) regressions incorporating data on starved and fed fish. In the linear example the HEf and HEm are estimated to be 60.88 and 93.66 kJ kgBW^{-0.8} d⁻¹, respectively. In the non-linear example the HEf and HEm are estimated to be 76.3 and 87.44 kJ kgBW^{-0.8} d⁻¹. The HEm estimated from the simultaneous prediction of the energetic efficiency of protein and lipid deposition using multiple linear regression was found to be 101.9±12.7 kJ kgBW^{-0.8} d⁻¹, however the standard error associated with this coefficient was high (Eq. 20). Although the corresponding values for HEf and HEm are quite different. This is to be expected because the estimate of HEm includes the additional heat production associated with the maintenance feeding ration. Assuming the swimming activity (HjE) of fish in both the fed and starved groups was similar, the difference between the HEf and HEm is indicative of the heat increment of feeding (HiE). If this is the case, then depending on the model used HiE accounts for 11.2 - 32.8 kJ kgBW^{-0.8} d⁻¹ or 12.8 - 35% of heat production associated with HEm in *S. lalandi*. A similar range of HiE values were found in *S. quinqueradiata* (Watanabe et al., 2000b).

The amount of DP required to maintain zero protein deposition in *S lalandi* was predicted to be 1.70 (Eq. 15) or 1.74 g DP kgBW^{-0.7} d⁻¹ (Eq. 17). These values are almost double that estimated for gilthead seabream (Lupatsch et al., 1998) and almost 5 times higher than found in white grouper reared at similar temperatures (Lupatsch & Kissil, 2005).

At present there is little other data on maintenance requirements for *S. lalandi*, however, Watanabe and co-authors have published several studies on *S. quinqueradiata* which provide useful comparisons. After scaling of their data the HEm for 750 g fish reared at temperatures between 12.8-16.5°C was estimated to be 77.5 kJ kgBW^{-0.8} d⁻¹ and maintenance protein requirements 1.37 g DP kgBW^{-0.7} d⁻¹ (Watanabe et al., 2000a). At temperatures between 21-25°C the HEm for 300-400 g fish was estimated to be 91.2 kJ kgBW^{-0.8} d⁻¹ and maintenance protein requirements 1.40 g DP kgBW^{-0.7} d⁻¹ (Watanabe et al., 2000b). Above 27°C HEm for fish weighing on average 80-180 g exceeded 138 kJ kgBW^{-0.8} d⁻¹ (Watanabe et al., 2000c). Our preferred estimate of HEm for *S. lalandi* is 87.4 kJ kgBW^{-0.8} d⁻¹, however this value is not dissimilar to the values derived from studies on *S. quinqueradiata* and confirms *Seriola spp*. have elevated maintenance requirements for energy and protein that exceed that of many cultured fish species.

Apart from estimating the efficiency of DE utilisation above maintenance, we did not consider using the linear regression which excluded starved fish to estimate or compare HEf or HEm (Eq. 14). Primarily this was because there was clearly a change in the efficiency of energy (and protein) utilisation above and below the HEm requirement when starved fish were included in the model. As a consequence the full data set was better described by a non-linear function and so extrapolation of Eq. 14 in order to predict HEf and HEm would be misleading. Similar curvilinear responses to energy or protein intake have been documented in other factorial studies on fish (Lupatsch et al., 1998; Glencross, 2008).

Perhaps one of the drawbacks to including data on starving fish in the respective models is that the efficiency of using energy from catabolism of body reserves such as glycogen or visceral organs in starving fish is occurring at a differential rate to that of fed fish, which are unlikely to mobilise body reserves to meet energy requirements. By default, the measured HEf under these conditions will be higher than the value extrapolated from fish receiving sufficient nutrients to satisfy or exceed their general HEm energy needs (i.e. from a linear regression). In this way van Milgen and Noblet (2003) estimated that the extrapolated HEf of fed pigs was approximately 62% of the measured value. Applying the same approach to our data the extrapolated intercept value of HEf in fed fish accounted for approximately 49% of the HEf in *S. lalandi* using the full data set (i.e. 29.7/60.9*100 = 0.49; Eq's 14 & 16). Interestingly, the intercept predicted from the linear regression of data that excluded starved fish predicted a HEf which was similar to the SMR of *S. lalandi* predicted for fish at 20°C and zero swimming velocity by Clark & Seymour (2006). If we also assume that the extrapolated value of 29.7 kJ kgBW^{-0.8} d⁻¹ represents HeE for a fish at rest and the value of 60.88 kJ kgBW^{-0.8} d⁻¹ includes the additional heat production associated with HjE, then an approximation of HjE can be made by difference. If this is the case then HjE accounts for almost 52% of HEf (31.9/60.88*100=0.52) or 34% of HEm (31.9/93.7*100=0.34).

4.6 Utilisation coefficients of DP or DE

Non-linear functions estimated that maximum protein or energy gain was achieved when DP or DE intake approached 6.22 g DP kgBW^{-0.70} d⁻¹ (Eq. 22) and 416 kJ DE kgBW^{-0.80} d⁻¹ (Eq. 23), respectively. However, these asymptotic values are clearly outside the range of our data and should be viewed cautiously. Actual maximum DP and DE intake in fish fed to apparent satiation were approximately 4.6 g DP BW(kg)^{-0.70} d⁻¹ and 238 kJ DE BW(kg)^{-0.80} d⁻¹, respectively. These values are slightly lower than the daily crude protein and gross energy intake reported in a New Zealand trial that examined the performance of S. lalandi reared on commercially available feeds (Moran et al., 2009). Use of the non-linear functions applied in this study implies that the efficiency of DP and DE utilisation changes as intake increases, being more efficient at low intakes and less so at higher intakes. This is also reflected in the increase in efficiency of DP and DE utilisation for models that include (Eq's 15 & 16) rather than exclude starving fish (Eq's 13 & 14). Most factorial studies to date have derived utilisation coefficients using linear models which incorporate data above and below maintenance rations, although problems with this approach have been discussed (Lupatsch et al., 1998; Glencross, 2008). Based on the functions derived from our full data set the utilisation coefficients for DP (0.51) and DE (0.65) are quite similar to those presented for mulloway (Pirozzi et al., 2010a), barramundi (Glencross, 2008) and a range of other marine species (Lupatsch et al., 2003a) including S. quinqueradiata (Watanabe et al., 2000b). However, from a production point of view the utilisation coefficients derived from the simple linear fits of data above maintenance are more practical as they are based on well fed fish reflective of real world situations. For this reason we selected 0.41 and 0.55 as the coefficients for the utilisation of DP or DE, respectively for use in our factorial model. Due to this approach our working values are probably slightly lower than reported for other species (Bureau et al., 2002).

Of major interest in this study was the evaluation of the partial energy costs of protein and lipid deposition (Eq. 24). We found the calculated energy cost to deposit protein (kJ per kJ protein deposited) was $1/K_P = 1.64\pm0.45$, while the energy cost to deposit lipid (kJ per KJ ipid deposited) was $1/K_L = 1.21\pm0.38$. The reciprocal values of $K_P = 0.61$ and $K_L = 0.83$ describe the energetic

efficiency with which protein or lipid were deposited. According to basic stoichiometry, the theoretical efficiency by which dietary protein (amino acids) or lipid are converted into tissue protein or tissue lipid is around 86% and 96%, respectively. Tissue lipid deposited from the conversion of amino acids is theoretically closer to 66% (Bureau et al., 2002). The energetic cost of lipid deposition is more efficient when it is supplied in the diet rather than by *de-novo* synthesis from non-lipid sources such as carbohydrates or amino acids (Bureau et al., 2002; Lupatsch et al., 2003a). More efficient transformation of dietary substrates into tissue also results in lower HiE. Our lipid coefficient was lower than the theoretical maximum for K_L and lower than K_L in species like European seabass, grouper and rainbow trout (i.e. 90%) (Bureau et al., 2002), suggesting that either protein-energy was involved in lipid deposition, as discussed by Lupatsch et al. (2003a), or that dietary carbohydrate-energy was involved in lipid deposition (Pirozzi, Booth & Allan, 2010b). The latter hypothesis is probably stronger considering our commercial feeds contained in excess of 20% NFE while the diets of Lupatsch et al. (2003a) consisted of fishmeal and fish oil. It is of interest to speculate whether feeding S lalandi diets that contain slightly more lipid and slightly less NFE may have improved the overall efficiency of energy retention via a reduction in the HiE associated with transformation of dietary CHO to lipid. This effect may have led to the slight improvements in feed conversion efficiency and weight gain seen in S. lalandi fed on diet COM B as it contained more dietary lipid and less NFE than diet COM A.

 K_P in *S. lalandi* was slight more efficient than K_P cited for other finfish species (e.g. 0.44-0.56) (Bureau et al., 2002; Lupatsch et al., 2003a; Pirozzi et al., 2010b) but not significantly so. Factors influencing K_P in fish a numerous and the individual or combined effects on K_P are not always clear. What is evident is that the values estimated in the majority of studies are generally much lower than the theory predicts and this has led to speculation that K_P has been considerably underestimated by *in-vivo* studies (Bureau et al., 2002). Variation in protein turnover, amino acid balance of diet and DP:DE ratio are all important factors. The fact our estimate of K_P was as high if not higher than the value of K_P cited for other species may indicate the feeds we evaluated were reasonably well balanced in terms of their amino acid profile and ratio of DP:DE.

4.7 *Diet specifications and predicted feeding rates*

Data on *S. lalandi* was combined to determine total daily protein and energy budgets for fish reared at 20-25°C (Table 5). Data was combined under a factorial approach that considers total energy requirement to be the sum of maintenance and growth such that,

$DE = M \cdot BW(kg)^b + G \cdot energy gained,$

where DE = digestible energy requirement in kJ fish⁻¹ d⁻¹; M = coefficient relating metabolic body weight to maintenance energy requirement in kJ BW(kg)^{-b} d⁻¹; G = coefficient predicting the efficiency of energy utilisation for growth and BW = body weight (Lupatsch et al., 2003a; Lupatsch, 2009). The same approach was used to determine total DP requirement. This allowed prediction of the changing DP:DE ratio with increasing fish size and subsequent estimates of feed intake and FCR for diets containing hypothetical levels of 12, 15 or 17 MJ DE kg⁻¹ (Table 5). These energy levels were selected because they represent common energy levels in commercial aquafeeds available in Australia (Pirozzi et al., 2010a) and New Zealand (Moran et al., 2009).

Two things are obvious from table 5; as *S. lalandi* grow their predicted DP:DE ratio declines and the amount of DE expended on maintenance requirement increases. Relative estimates of the amount of DE expended on overall maintenance energy needs range from 25 to 34% of total DE intake in fish weighing between 50 to 2000 g, respectively. These responses are well documented in fish (Lupatsch & Kissil, 2005; Glencross, 2008; Pirozzi et al., 2010a) and are symptomatic of the decline in predicted feed conversion efficiency as fish size increases. The DP:DE requirement of *S. lalandi* and the proportion of energy expended on maintenance is notably higher than for other carnivorous species at the same weight such as mulloway, barramundi or white grouper reared at similar temperatures (Lupatsch & Kissil, 2005; Glencross, 2008; Pirozzi et al., 2010a). This is

understandable given the high maintenance requirement of *S. lalandi* and the pragmatic coefficient we selected for the utilisation of DP.

Piecewise regression analysis as applied to our data set indicated there were 3 key growth stages in the production of *S. lalandi*; a juvenile stage up to 200g, an intermediate stage between 200-1000g and a later stage for fish growing above 1000g (Fig. 6). This analysis is extremely useful in deciding which diet specifications are the most appropriate for each production stage, because although the factorial model is capable of estimating DP:DE requirement for any size animal it is unlikely that a feed manufacturer would elect to produce more than 3 or 4 specific feeds for any one species. In addition, the manufacture of feeds containing very high theoretical levels of DP is not generally possible once other dietary constituents such as lipids and binders are included and inclusion limits are placed on certain ingredients. High levels of DP also tend to drive up the DE value of a feed so successful formulation of high DP - low DE diets can be difficult. Of equal importance is consideration of potential feed intake and the factors that may limit nutrient intake such as excessive dietary energy level, adverse husbandry practices (e.g. poor feeding regimes) or environmental influences such as temperature. Lastly, there is the matter of feed cost and biological and economic FCR.

We have decided to recommend feeds by selecting an intermediate DP:DE ratio for each of the objectively identified growth stages; namely 38, 31 and 24g DP MJDE⁻¹, respectively. This has the effect of averaging the specification over the growing range. In addition, we have assumed that the highest practically achievable level of DP for a manufactured feed is \leq 550g kg⁻¹ diet. A DP:DE of 38g DP MJDE⁻¹ is naturally for small, fast growing fish up to 200g. Therefore, the type of diet that would best meet these constraints is one that contains about 456g DP kg⁻¹ and 12 MJ DE kg⁻¹ (Table 6). This is the specification that will also deliver the best theoretical FCR at that energy level. The lower DE of this diet means a higher relative feed intake is required, but this is well within the normal intake range recorded for S. lalandi reared at our experimental temperature (Fig. 4). For fish growing between 200 - 1000g a diet containing 465g DP kg⁻¹ and 15 MJ DE kg⁻¹ would be suitable while a diet containing 432g DP kg⁻¹ and 18 MJ DE kg⁻¹ would be appropriate for fish > 1000g. These recommendations are highlighted in the shaded sections of Table 6. It may be possible to manufacture diets with DP contents approaching 558 to 570 g DP kg⁻¹ (e.g. unshaded sections of table 6) which would lead to obvious improvements in FCR, however these diets would have to contain a mix of expensive, highly digestible protein and energy dense ingredients similar to or better than fish meal.

5. CONCLUSIONS

This study used a factorial approach to estimate the DP and DE requirements of *S. lalandi* reared at $20-25^{\circ}$ C and weighing between 50 to 2000g. Estimates of the DP and DE requirements of *S. lalandi* were subsequently used to calculate theoretical changes in the optimum ratio of DP:DE (g DP MJ DE⁻¹) with increasing fish size, allowing prediction of theoretical dietary DP content, FCR and feed requirements for diets containing different levels of DE. Our research has demonstrated that the DP and DE requirements of *S. lalandi* are high compared to other carnivorous fish species due to the fact that *S. lalandi* appear to use a considerable proportion of the DP they consume as an energy source rather than for tissue synthesis. This is supported by the proportionally high amount of DP and DE required to meet maintenance demands.

The factorial model we present has been determined at water temperatures that are close to the optimal water temperature reported for this species (Pirozzi & Booth, 2009). As such a greater understanding of the effects of temperature on potential growth rate, maintenance requirements and utilisation coefficients will improve the usefulness of this model, especially as the majority of the aquaculture production of *S. lalandi* currently takes place in South Australia where water temperature fluctuates dramatically between winter $(12^{\circ}C)$ and summer $(24^{\circ}C)$. In Australia, *S lalandi* are generally harvested when they attain 4-6kg live weight, so further improvement of the model will require new information on growth rate and compositional analysis of larger fish.

Finally, the predicted requirements and the iterative feed specifications derived from this model should be validated in order to determine its accuracy and reliability.

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	Diet typ	pe ¹
Nutrient	Com A	Com B
Gross nutrient content		
Crude protein (g kg ⁻¹)	530.9 + 0.4	511.9 ± 0.0
Fat $(g kg^{-1})$	110.5 ± 2.1	162.0 ± 1.4
$Ash(g kg^{-1})$	106.0 ± 1.4	111.0 ± 0.0
NFE^2 (g kg ⁻¹)	252.6 ± 3.1	215.1 ± 1.4
Phosphorous $(g kg^{-1})$	14.2 ± 0.1	15.3 ± 0.2
Gross energy (MJ kg ⁻¹)	21.4 ± 0.0	22.1 ± 0.0
Digestible nutrient content ^{3,4}		
Digestible protein (g kg ⁻¹)	316.2 ± 0.7	363.9 - 384.3
Digestible fat (g kg ⁻¹)	62.7 ± 1.1	100.5 - 101.2
Digestible phosphorous (g kg ⁻¹)	4.0 ± 0.3	3.7 - 5.5
Digestible energy (MJ kg ⁻¹)	10.8 ± 0.0	13.1 - 14.5
DP:DE ratio	29.3	27.0

Gross and digestible nutrient composition of commercial feeds fed to S. lalandi at a temperature of $23.7 \pm 0.7^{\circ}$ C. Data values are mean \pm sem (dry matter basis).

¹ Gross nutrient content of diets analysed in duplicate
² By difference; NFE = (1000 - ash - crude protein - fat)
³ Digestible nutrient content = ADC% x gross nutrient or energy content
⁴ Digestible data values for COM B are for individual replicates

Performance of different size class *S. lalandi* starved for a period of 12 days at a temperature of 21.0 ± 2.2 °C. Data values are for each cage.

Start weight (g)	Harvest weight (g)	Weight loss (g fish ⁻¹ d ⁻¹)	GMBW (g)	Protein loss (g fish ⁻¹ d ⁻¹)	Lipid loss (g fish ⁻¹ d ⁻¹)	Energy loss (kJ fish ⁻¹ d ⁻¹)	
39.80 40.30 41.65	29.00 30.10 31.30	0.90 0.85 0.86	33.97 34.83 36.11	0.23 0.18 0.19	0.057 0.053 0.057	9.44 7.61 7.37	
85.90 86.70 88.40 193.50	73.40 77.50 167.50	0.92 1.11 0.91 2.17	80.21 79.77 82.77 180.03	0.20 0.23 0.18 0.44	0.158 0.185 0.155 0.340	10.94 16.15 12.40 27.03	
213.25 242.25 340.25	187.10 228.40 315.40	2.18 1.15 2.07	199.75 235.22 327.59	0.46 0.27 0.52	0.229 0.350 0.628	14.30 20.70 39.55	
349.73 365.25 428.30 467.30 601.00	340.70 396.20 443.90 556.00	2.24 2.05 2.68 1.95 3.75	352.76 411.94 455.45 578.06	0.22 0.31 0.85 0.78 0.85	0.553 1.521 0.962 1.665	43.49 31.88 58.58 58.00 57.15	

Variation in essential (EAA) and non-essential (NEAA) amino acid composition of different size *S. lalandi*; data presented on a wet basis ($g kg^{-1}$) or as percent of crude protein content.

Fish wt	53	90	302	580	53	90	302	580	
Crude protein (%)	18.40) 19.44	19.58	19.90	_	_	-	-	_
Ash (%)	3.71	3.54	3.37	2.92	-	-	-	-	_
Fat (%)	3.10) 3.92	5.11	9.18	-	-	-	-	-
G. Eenrgy (MJ kg ⁻¹)	5.44	5.99	6.50	7.85	-	-	-	-	-
-	EAA	$(g kg^{-1})$	wet basi	s)	EAA (%	6 crude	e prote	in)	EAA(ave±sd)
Arginine	13.99	14.75	14.80	14.95	7.61	7.59	7.56	7.51	7.57±0.04
Histidine	6.95	7.71	8.58	8.54	3.78	3.97	4.38	4.29	4.10±0.28
Iso-Leucine	6.65	6.96	7.38	7.61	3.61	3.58	3.77	3.82	3.70±0.12
Leucine	11.23	11.77	12.11	12.62	6.10	6.05	6.18	6.34	6.17±0.13
Lysine	10.41	10.44	11.57	10.84	5.66	5.37	5.91	5.45	5.60±0.24
Methionine	4.50	4.77	4.64	4.76	2.44	2.45	2.37	2.39	2.41±0.04
Phenylalanine	6.52	7.06	7.10	7.52	3.54	3.63	3.63	3.78	3.64±0.10
Threonine	7.69	8.24	8.64	8.80	4.18	4.24	4.41	4.42	4.31±0.12
Tryptophan	1.63	1.78	1.78	1.90	0.89	0.92	0.91	0.96	0.92 ± 0.03
Valine	7.49	7.84	8.42	8.42	4.07	4.03	4.30	4.23	4.16±0.13
	NEA.	A (g kg⁻ A(ave±s	¹ wet band	usis)	NEAA		(%	C	rude protein)
		1(4+0-2	<i>(</i> u)						
Alanine	9.74	10.09	10.80	10.37	5.30	5.19	5.51	5.21	5.30±0.15
Aspartic acid	11.92	12.15	13.12	12.24	6.48	6.25	6.70	6.15	6.39±0.25
Cystine	1.74	1.82	1.69	1.70	0.95	0.94	0.86	0.86	0.90 ± 0.05
Glutamic acid	18.74	18.93	20.40	18.87	10.19	9.74	10.42	9.48	9.95±0.42
Glycine	14.69	15.55	15.31	15.15	7.98	8.00	7.82	7.61	7.85±0.18
Proline	8.45	9.23	8.90	8.75	4.59	4.75	4.54	4.40	4.57±0.14
Serine	7.17	7.59	7.76	7.59	3.90	3.90	3.96	3.81	3.89±0.06
Tyrosine	5.41	5.86	6.22	6.22	2.94	3.02	3.18	3.13	3.07±0.11

*Non essential AA's asparagine and glutamine were not determined.

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Feeding ¹ treatment	Stock wt (g)	Harvest wt (g)	Feed intake (g fish ⁻¹ day ⁻¹)	FCR	DP ² intake (g fish ⁻¹ d ⁻¹)	Protein gain (g fish ⁻¹ d ⁻¹	DE ² intake) (kJ fish ⁻¹ d ⁻¹)	Energy gain (kJ fish ⁻¹ d ⁻¹)	DL ² intake (g fish ⁻¹ d ⁻¹)	Lipid gain (g fish ⁻¹ d ⁻¹)
COM A L 25	229.80	294.50	3.52	1.91	1.11	0.46	37.88	15.37	0.22	0.12
COM A L 50	229.10	376.90	6.45	1.53	2.04	0.91	69.32	31.78	0.40	0.34
COM A L 75	222.20	445.50	9.37	1.47	2.96	1.36	100.75	51.36	0.59	0.59
COM A L Sat	225.80	490.10	12.09	1.60	3.82	1.51	129.91	65.99	0.76	0.86
	116.67	152 75	2.24	2 10	0.71	0.21	24.00	5 14	0.14	0.01
COM A S 25	111.00	192.75	2.24	2.19	0.71	0.21	24.09	12.26	0.14	0.01
COM A S 50	111.00	181.92	5.98	1.97	1.20	0.38	42.81	12.20	0.25	0.08
COM A S /S	118.75	245.08	5.72	1.59	1.81	0.74	61.52	26.18	0.36	0.27
Com A S Sat	114.17	261.83	7.10	1.68	2.24	0.81	76.28	33.31	0.44	0.40
COM B L 25	214.20	307.40	4.14	1.56	1.55	0.55	57.16	23.69	0.42	0.26
COM B L 50	220.00	412.20	7.69	1.40	2.88	1.21	106.04	50.66	0.78	0.65
COM B L 75	222.50	483.90	11.19	1.50	4.18	1.59	154.26	72.34	1.13	1.06
COM B L Sat	233.80	600.30	14.56	1.39	5.45	2.20	200.71	103.19	1.47	1.46
COMPS 25	111 17	150 (7	2 (4	1.02	0.00	0.27	26.25	0.05	0.27	0.07
COM B S 25	111.17	159.67	2.64	1.93	0.99	0.27	36.35	9.05	0.27	0.07
COM B S 50	108.42	226.42	4.77	1.42	1.79	0.65	65.81	26.93	0.48	0.32
COM B S 75	117.75	288.00	6.91	1.42	2.58	1.12	95.26	46.42	0.70	0.57
COM B S Sat	118.17	315.67	8.70	1.56	3.25	1.10	119.97	50.70	0.88	0.68

Average performance of different size class S. lalandi fed decreasing rations of COM A or COM B for 35 days at a temperature of $23.1 \pm 1.4^{\circ}$ C

Note: tabulated values are average of duplicate replicate tanks.

¹ Abbreviations; COM A = commercial diet A, COM B = commercial diet B, L = large fish; S = small fish, Sat = fed to apparent satiation. ² Digestible nutrient and energy intake of fish fed COM B based on mean of data presented in Table 1. Initial wet basis composition of small size class; moisture=75.2%, crude protein=18.5%, gross energy=5.1 MJ kg⁻¹, fat=2.4%, ash=3.7%. Initial wet basis composition of large size class; moisture=74.9%, crude protein=18.8%, gross energy=5.3 MJ kg⁻¹, fat=3.0%, ash=3.2%

Estimate of digestible protein and energy requirements of S. lalandi reared at 21-24°C.

						<u>``</u>		
					Fish weight (g	5)		
	50	100	250	500	750	1000	1500	2000
1. Maximum weight gain (g fish ⁻¹ d ⁻¹)	2.46	3.56	5.82	8.45	10.50	12.25	15.23	17.77
<u>DE Requirements</u> 2 Metabolic body weight	0.09	0.16	0 33	0.57	0 79	1.00	1 38	1 74
3. Maintenance energy requirement (kJ fish ⁻¹ d ⁻¹)	7.96	13.86	28.84	50.22	69.46	87.44	120.94	152.24
4. Predicted energy content of fish $(kJ g^{-1})$	5.16	5.76	6.65	7.41	7.90	8.27	8.81	9.22
5. Predicted energy gain of fish (kJ fish ⁻¹ d^{-1})	12.68	20.51	38.73	62.63	82.97	101.29	134.19	163.82
6. Productive energy requirement (kJ fish ⁻¹ d^{-1})	23.21	37.53	70.87	114.61	151.84	185.37	245.57	299.80
7. Total daily energy requirement (kJ fish ⁻¹ d ⁻¹)	31.17	51.39	99.71	164.84	221.30	272.81	366.51	452.04
8. Energy expended on maintenance (%)	25.54	26.97	28.93	30.47	31.39	32.05	33.00	33.68
<u>DP Requirements</u>								
9. Metabolic body weight	0.12	0.20	0.38	0.62	0.82	1.00	1.33	1.62
10. Maintenance protein requirement (g fish ⁻¹ d^{-1})	0.21	0.34	0.64	1.05	1.39	1.70	2.26	2.76
11. Predicted protein gain of fish (g fish ⁻¹ d ⁻¹)	0.46	0.67	1.10	1.59	1.98	2.31	2.87	3.34
12. Productive protein requirement (g fish ⁻¹ d^{-1})	1.14	1.65	2.70	3.92	4.87	5.68	7.07	8.24
13. Total daily protein requirement (g fish ⁻¹ d ⁻¹)	1.35	1.99	3.35	4.97	6.26	7.38	9.32	11.01
14. Predicted dietary DP:DE (g DP MJ DE ⁻¹)	43.3	38.8	33.6	30.1	28.3	27.1	25.4	24.3

1. Maximum weight gain (g fish⁻¹ d⁻¹) = $0.3011 \cdot BW(g)^{0.5365}$; Eq.6 2. Metabolic body weight for energy; $BW(kg)^{0.8}$

3. Maintenance energy requirement (kJ fish⁻¹ d⁻¹) = 87.44•BW(kg)^{-0.80} d⁻¹; Eq. 21 4. Predicted energy content of fish (kJ g⁻¹) = 2.79•BW(g)^{0.157}; Eq. 11 5. Predictive energy gain (kJ fish⁻¹ d⁻¹) = (2.79•BW(g)^{0.157})•maximum daily weight gain; Eq's. 10 & 11 6. Productive energy requirement (kJ fish⁻¹ d⁻¹) = 1.83•predicted energy gain

7. Total daily energy requirement (kJ fish⁻¹ d⁻¹) = maintenance energy requirement + productive energy requirement

8. Percent energy expended on maintenance = (maintenance energy requirement / total energy requirement)•100

9. Metabolic body weight for protein; $BW(kg)^{0.7}$

10. Maintenance protein requirement (g fish⁻¹ d⁻¹) = $1.7 \cdot BW(kg)^{-0.70} d^{-1}$; Eq. 20

11. Predicted protein gain (g fish⁻¹ d⁻¹) = 18.82/100·maximum daily weight gain; Eq's. 6 & 10

12. Productive protein requirement (g fish⁻¹ d⁻¹) = 2.46•predicted protein gain

13. Total daily protein requirement (g fish⁻¹ d⁻¹) = maintenance protein requirement + productive protein requirement

14. Predicted DP:DE = total protein requirement (g)/(total energy requirement (kJ) \cdot 1000)

Iterative feed specifications and associated feed requirements for S. lalandi fed 12, 15 or 18 MJ kg⁻¹ diets and reared at 21-24°C. Shaded boxes indicate possible practical diet specifications for different growth stages.

					Fish weight (g	g)		
	50	100	200	300	600	900	1000	2000
Dietary DP:DE (g DP MJ DE ⁻¹)	38	38	38	31	31	31	24	24
<u>12 MJ DE kg⁻¹ diet</u> Estimated DP content of diet (g kg ⁻¹) Feed requirement (g fish ⁻¹ d ⁻¹) Feed requirement (% BW ⁻¹ d ⁻¹) Expected FCR	456.0 2.60 5.19 1.06	456.0 4.28 4.28 1.20	456.0 7.07 3.53 1.37	372.0 9.48 3.16 1.48	372.0 15.68 2.61 1.68	372.0 21.06 2.34 1.82	288.0 22.73 2.27 1.86	288.0 37.67 1.88 2.12
<u>15 MJ DE kg⁻¹ diet</u> Estimated DP content of diet (g kg ⁻¹) Feed requirement (g fish ⁻¹ d ⁻¹) Feed requirement (% BW ⁻¹ d ⁻¹) Expected FCR	570.0 2.08 4.16 0.85	570.0 3.43 3.43 0.96	570.0 5.66 2.83 1.09	465.0 7.59 2.53 1.18	465.0 12.54 2.09 1.35	465.0 16.85 1.87 1.45	360.0 18.19 1.82 1.48	360.0 30.14 1.51 1.70
<u>18 MJ DE kg⁻¹ diet</u> Estimated DP content of diet (g kg ⁻¹) Feed requirement (g fish ⁻¹ d ⁻¹) Feed requirement (% BW ⁻¹ d ⁻¹) Expected FCR	684.0 1.73 3.46 0.71	684.0 2.86 2.86 0.80	684.0 4.71 2.36 0.91	558.0 6.32 2.11 0.98	558.0 10.45 1.74 1.12	558.0 14.04 1.56 1.21	432.0 15.16 1.52 1.24	432.0 25.11 1.26 1.41

Feed requirement (g fish⁻¹ d⁻¹) = total daily energy requirement/ DE content of diet (from Table 5) Feed requirement (% BW⁻¹ d⁻¹) = feed requirement (g)/BW(g)*100 FCR = feed requirement (g fish⁻¹ d⁻¹) / weight gain (g fish⁻¹ d⁻¹) DP content of diet (g kg⁻¹) = DP:DE ratio•DE content of diet



Relationship between geometric mean body weight and daily protein (a) or daily energy loss (b) in *S. lalandi*. Regressions are iteratively fit to data using the power function $y = a \cdot BW(kg)^b$.



Curvilinear relationship between protein deposition and digestible protein intake (a) or energy deposition and digestible energy intake (b) for *S. lalandi*.



Relationship between daily growth rate and geometric mean body weight; outer curves are 95% prediction bands (a) and between body weight and fork length (b) of *S. lalandi*.



Relationship between relative feed intake and geometric mean body weight of *S. lalandi* reared in different experiments at temperatures $> 20^{\circ}$ C and fed to apparent satiation.



Wet basis chemical and gross energy content of whole *S. lalandi* weighing from 9 - 1360 g; % moisture content (a), % dry matter content (b), % protein content (c), energy content kJ g⁻¹ (d), % fat content (e), % ash content (f). Refer to text for models and parameter estimates.



Requirements of DP:DE in growing *S. lalandi* reared at 20-25°C. Theoretical model based on data from table 5; DP:DE ratio = $79.21 \cdot BW(g)^{-0.1522}$. Breakpoints estimated using linear-linear piecewise regression analysis.

4.14 Glycaemic response of juvenile yellowtail kingfish (Seriola lalandi) following an intraperitoneal or oral administration of D-glucose.

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ABSTRACT

Yellowtail kingfish Seriola lalandi are a pelagic marine carnivore and an exciting new prospect in Australian aquaculture. At present, commercial feeds for this species contain low levels of carbohydrate due to the perception that *Seriola spp.* is incapable of digesting and metabolising high levels of carbohydrate for energy purposes. The current study describes two experiments that investigate and re-evaluate the metabolism of carbohydrate by this species. The first experiment evaluated the uptake and clearance of glucose from the blood following an acute intra-peritoneal injection of 1g D-glucose kg BW⁻¹. The second experiment evaluated the uptake and clearance of glucose from the blood following an acute oral dose of 1, 3 or 6g D-glucose kg BW⁻¹. Plasma glucose level peaked at 12.8 mM between 1 and 2 h after intra-peritoneal injection and yellowtail kingfish experienced prolonged hyperglycaemia for 12 h. In fed fish, the peak plasma response reflected the increase in the concentration of glucose contained in the diet, increasing to approximately 10.8, 14.4 and 22.9 mM in fish fed the 1, 3 or 6g D-glucose kg BW⁻¹, respectively. However, despite the increasing concentration of glucose the peak response time was similar and occurred approximately 6h after ingestion. The oral intake of glucose also extended the period of hyperglycaemia to between 18-24 h. Glycaemic response to dose rate was quantified by calculating the area under each response curve (AUC). The effect of dose rate was highly significant on AUC, with values of 72.0, 120.6 and 208.6 mMh recorded in fish fed the 1, 3 or 6g D-glucose kg BW⁻¹ treatments, respectively. Previously regarded as extremely intolerant of carbohydrate, results from the current study suggest yellowtail kingfish are able to absorb and clear an injected or oral glucose load more efficiently than many other carnivorous teleosts and exhibit a clearance response that is not dissimilar to many omnivorous fish species. This may indicate that yellowtail kingfish is capable of utilising higher levels of carbohydrate in their feeds than is currently accepted.

1. INTRODUCTION

Plant-based protein and energy sources are being increasingly used in commercial aquafeeds. However, while many plant ingredients are attractive in terms of their amino acid profiles or gross energy content, the majority of them are high in carbohydrate or indigestible fibre, which is problematic for many fish species (Wilson, 1994; Hemre et al., 2002), especially carnivores such as Seriola spp. which are reported to be intolerant of dietary carbohydrates such as starch and glucose (Furuichi et al., 1986). Glucose itself is one of the major end products of carbohydrate digestion and a major potential source of energy for most terrestrial animals. However its role in fish nutrition is not as well understood and remains the subject of debate (Wilson, 1994; Stone, 2003a). A rapid and effective method of examining glucose tolerance in fish is via a glucose tolerance test (GTT) where glucose is administered to individual fish orally, intravenously or intra-peritoneally and the uptake and clearance of from the bloodstream is monitored over time. Previous studies have demonstrated that when glucose is administered as an acute dose, the majority of fish species exhibit an intense and prolonged state of hyperglycaemia. This has led to fish being regarded as generally intolerant to dietary glucose when compared to mammals (Wilson, 1994; Moon, 2001; Hemre et al., 2002; Stone, 2003a; Enes et al., 2009). However, this intolerance is species-specific and is usually related to the natural trophic level of the species, with herbivorous and omnivorous fishes generally being more efficient at using glucose than strictly carnivorous species (Furuichi & Yone, 1981; Wilson, 1994; Peres et al., 1999; Legate et al., 2001). Despite this general trend, species specific research must be carried out because large variations in glucose tolerance exist even within trophic level.

A poorly understood area in the utilisation of dietary carbohydrate by fish concerns the various mechanisms involved in the facilitation of glucose transport into the bloodstream following digestion and also the subsequent utilisation of glucose for metabolic purposes. Several hypotheses for this general intolerance have been discussed and include higher sensitivities of insulin to amino acids rather than glucose (Moon, 2001; Hemre et al., 2002), inefficiencies in peripheral glucose utilisation and absorption (Moon, 2001; Wright et al., 2000) and also inadequacies in homeostatic glucose regulation and the imbalance between endogenous and exogenous glucose sources (De Silva & Anderson, 1994; Wilson, 1994; Enes et al., 2008; Kirchner et al., 2008).

The use of glucose as a fuel during periods of high activity in high performance verses sedentary fish species has been found to vary significantly (Weber & Haman,1996). As glucose is a major source of ATP-derived energy available during sprint swimming, several adaptations are believed to occur in high performance fish which may allow higher glucose turnover and utilisation rates. These adaptations include possible higher intracellular glycogen stores, increased densities of glucose transporters and an increase in intra-muscular hexokinase activity (Weber & Haman, 1996). Due to unreliable or sporadic supplies of exogenous glucose in the natural environment, sprint- or high performance swimming in fish is largely dependent on closed white muscle systems and energy is derived from the anaerobic breakdown of intracellular glycogen (Weber & Haman, 1996). The ability to utilise stored glycogen via glycogenolysis and increased hexokinase function during peak activity may allow fish to bypass any limitations involved in the extracellular transport of glucose.

Yellowtail kingfish *Seriola lalandi* is a high performance pelagic marine carnivore and a relatively new species in the Australian aquaculture industry. It has huge potential in both domestic and international markets (Love & Langenkamp, 2003). Yellowtail kingfish are reared in sea-cages and fed extruded pellets containing 45-50% crude protein, 15-20% fat and 17-20 MJ kg⁻¹. These diets are low in carbohydrates such as wheat which is primarily included as a binding agent. Higher levels of dietary carbohydrate may be possible, which would allow feed manufacturers much greater flexibility with ingredient formulation, but there is a perception that yellowtail kingfish would respond poorly to feeds containing too much carbohydrate.

This study describes two experiments designed to improve our understanding of carbohydrate utilisation in juvenile yellowtail kingfish. The first experiment evaluated the ability of yellowtail kingfish to assimilate and clear an acute load of glucose from the bloodstream following an intraperitoneal injection of 1g D-glucose kg BW⁻¹. This experiment is hereafter known as the intraperitoneal glucose tolerance test (IP test). The second experiment studied the response of yellowtail kingfish to diets that contained different amounts of D-glucose (1, 3 and 6g D-glucose kg BW⁻¹). This experiment is hereafter known as the oral glucose tolerance test (Oral test).

2. MATERIALS AND METHODS

2.1 Fish, facilities and stock solution

Fish for use in both experiments were progeny of wild-caught yellowtail kingfish brood-stock held at Industry & Investment NSW Port Stephens Fisheries Institute (PSFI), NSW, Australia. Fish used in the IP test experiment had a mean weight \pm sd of 228.3 \pm 3.4g (n= 138) and fish used in OGTT experiment had a mean weight \pm sd of 286.9 \pm 4.6g (n=132).

Prior to the experiments all fish were housed in a 10kL holding tank and fed a high protein, low carbohydrate commercial marine finfish diet (Skretting Australia Pty. Ltd, Cambridge, Tasmania, Australia; reported nutrient composition - crude protein 50%, crude lipid 17%, gross energy 21 MJ kg⁻¹).

Both experiments were performed using individual fish housed in circular 200L floating cages. Floating cages were fitted with lids and constructed of 9mm plastic mesh and lined with solid black vinyl to reduce internal and external disturbance of fish. Experiment cages were secured in large

10kL tanks capable of holding 10-12 cages around the perimeter. The 10kL tanks were connected to a saltwater recirculating aquaculture system (RAS) that supplied filtered estuarine water to each 10kL tank at a flow rate of approximately 70L min⁻¹, however during experiments all flows were stopped. The 10kL tanks were constantly aerated via a large central air-stone which ensured gentle movement of well aerated water around and through the experiment cages. To assist in overnight blood sampling and to minimise stress to fish, fluorescent lighting was controlled to give a 24L:0D photoperiod for the duration of the IP experiment whilst photoperiod was controlled to 12L: 12D during the oral experiment.

2.2 *IP test - experimental design and procedures*

The IP experiment was designed to record temporal changes in the plasma glucose concentration of yellowtail kingfish that had been given an intra-peritoneal injection of 1g D-glucose kgBW⁻¹. A stock solution of D-glucose was prepared by dissolving 50g analytical reagent grade D-glucose (D-(+)- glucose 99.5%, SIGMA-Aldrich Pty. Ltd. Castle Hill, NSW, Australia) in 100ml of sterilised (autoclaved) distilled water to give a standard 0.5g mL⁻¹ glucose solution. Two procedural controls were used; fish given a sham injection of a similar volume of 0.9% sodium chloride (saline solution; AstraZeneca) and fish handled in a similar way to all other fish but not given an IP injection. Blood samples were withdrawn 0 (baseline), 1, 2, 3, 6, 9, 12, 18, 24, 48 or 72 hours after initial injection or handling treatments. All fish were only sampled once.

Prior to the experiment fish were lightly anaesthetised (10mg Aqui-S L⁻¹; Aqui-S New Zealand Ltd, Lower Hutt N.Z), randomly selected and transferred from the 10kL holding tank into 4 smaller holding tanks filled with well aerated saltwater ($0.5m^3$). Each holding tank contained ≈ 40 fish. Subsequently, the fish in one of the holding tanks were anaesthetised, individually weighed and randomly assigned to one of the experimental treatments. The treatment was then administered, the time was recorded and the fish was transferred into a randomly selected experiment cage to recover and await blood sampling. This process was repeated with each group of fish over consecutive days providing n=4 replicate data points for each treatment. Prior to administering treatments, the blood of 4 fish from each consecutive group was sampled in order to provide a resting plasma glucose level (i.e. 0 h).

At the appropriate time individual fish were captured without anaesthetic and placed upside down in a split, soft foam block lined with plastic in order to expose the ventral surface. Blood was withdrawn form the caudal vein (<1mL) of un-anaesthetised fish using a 23 gauge x 1.25mm hypodermic needle and 3mL syringe (Becton-Dickinson B-D, Singapore). All blood samples were collected within 60 seconds of initial capture to minimise the risk of stress-induced changes in blood glucose concentration (Stone et al. 2003b; Booth et al. 2006). Following blood sampling, fish were removed from the experiment and transferred to a separate holding tank to recover.

2.3 Oral test - experimental design and procedures

The oral test experiment was designed to record temporal changes in the plasma glucose concentration of yellowtail kingfish fed different amounts of a diet containing 40% by weight of D-glucose (D-(+)- glucose 99.5%, SIGMA-Aldrich Pty. Ltd. Castle Hill, NSW, Australia). Three dose rates were used to study the effect of concentration; 1, 3 or 6g D-glucose kgBW⁻¹. The amount of D-glucose administered was controlled by restrictively feeding pre-weighed individual fish a predetermined amount of feed. A control group was established by feeding a diet formulated to be low in dietary carbohydrate content. The formulation of the glucose and control diet is presented in Table 1. Each of the treatments was administered to 3 different groups of fish in 3 separate runs performed under similar circumstances (n=3).

Each of the 2 diets was manufactured using a small scale meat mincer fitted with a 6mm pellet die (Barnco Australia Pty. Ltd., Leichardt, NSW, Australia). Prior to mixing, all ingredients were ground in a hammermill fitted with 1.5mm screen (C-E Raymond Inc. IL, USA). Batched diets were

then thoroughly dry mixed (Hobart mixer; Hobart Corporation, Troy, OH, USA) before distilled water was added to form each mash into a wet dough. Each dough was then cold pressed into pellets which were dried in a convection drier at $<35^{\circ}$ C for approximately 7 hours. Prior to and during the experiment both diets were stored frozen at $<-15^{\circ}$ C.

Prior to the experiment fish were lightly anaesthetised (10mg Aqui-S L⁻¹; Aqui-S New Zealand Ltd, Lower Hutt N.Z), randomly selected and transferred from the 10kL holding tank into 3 smaller holding tanks filled with well aerated saltwater (0.2m³). Each holding tank contained ≈ 45 fish. Subsequently, the fish in one of the holding tanks were anaesthetised, individually weighed and randomly distributed to one of the experiment cages in preparation for feeding. Following stocking each fish was randomly assigned to one of the treatments (i.e. 1, 3 or 6g D-glucose kg BW^{-1} or the control diet) and fasted for 48 hours. Prior to feeding, the blood of 3 fish was collected to record resting plasma glucose concentration (i.e. 0h). The predetermined ration for each of the glucose treatments was then fed to the appropriate fish and the time of feeding recorded. Yellowtail kingfish assigned to the control treatment were fed the same ration by weight of fish assigned to the 6g Dglucose kg BW-1 treatment because we assumed that any effect of the control diet on plasma glucose level would be most pronounced at the highest feed intake level. Blood samples were subsequently taken from individual fish 1, 2, 3, 6, 9, 12, 18, 24, 36 or 48 hours post-feeding using similar methods to that employed in the IP test. Blood was sampled from each fish only once after which they were transferred to a separate holding tank to recover. The process was repeated with each group of fish over consecutive days providing n=3 replicate data points for each treatment. Prior to feeding, the blood of 3 fish from each consecutive group was sampled in order to provide a resting plasma glucose level (i.e. 0 h).

2.4 Measurement of blood glucose

Blood samples were immediately analysed for plasma glucose (Kitchener & Freitas 2008) using a calibrated Accu-chek performa® hand-held blood glucose meter (Roche Diagnostics 2006 Australia, Castle Hill, NSW, Australia). The Accu-chek Performa® uses an enzymatic reaction whereby the enzyme (dehydrogenase) on each test strip in the presence of the co-enzyme Pyrroloquinoline quinone (PQQ) creates a small DC electrical current when reacting with the amount of glucose in each blood sample (Roche Diagnostics 2006). The meter is then able to interpret the strength of this electrical current and convert it directly to a plasma glucose reading in mM (Roche Diagnostics 2006). Prior to the study the glucose meter was calibrated to a single batch of test strips according to the manufacturer's instructions. One test strip was used for each fish.

2.5 Water quality in IP test and oral test

During both experiments, pH (7-8), salinity (29.7-30.1 ppt), temperature (18.7-21.1 °C) and dissolved oxygen (> 6 mg L⁻¹) in experimental tanks were measured daily using a Horiba U10 water quality analyser (Horiba, Japan). Total ammonia for both experiments was measured at the commencement of each experimental run using a rapid colourmetric method (<0.6mg L⁻¹) (E.Merck, Model 1.08024, Germany).

2.6 Statistical analysis

One-way analysis of variance (*ANOVA*) was used to examine the effect of treatment on the plasma glucose concentration of yellowtail kingfish at each progressive time point and Duncan's multiple comparisons procedure was used to discriminate between treatment means if *ANOVA* was significant. Alpha for both tests was set at 0.05.

A separate Area Under Curve (AUC) analysis was performed on the glucose response curves of all treatments (i.e. per run per experiment) using the trapezoidal method in GraphPad Prism V.5 software package (GraphPad Software Inc. La Jolla, California, U.S.A). AUC analysis was performed after setting the following parameters: baseline values were defined as the mean of the

first and the last two time points in each treatment; peaks less than 10% the difference between minimum and maximum plasma glucose values and with fewer than three adjacent points were ignored and all negative peaks were ignored. Following AUC analysis, one-way *ANOVA* was used to compare treatment means of total incremental area under the glucose response curves (AUC), peak glycaemic response (Y_{max}), time at peak glycaemic response (X_{max}) or time when the curve returned to the baseline (X_{final}). Post-hoc comparisons between means were performed using Duncan's multiple comparisons procedure.

Prior to *ANOVA* analysis, raw data were subjected to Cochran's test to confirm the assumption of homogeneous variances. Where necessary, raw data was log transformed to meet that assumptions. All *ANOVA* analysis was performed using Statgraphics Plus for Windows (Manugistics, Inc. Rockville, Maryland, U.S.A). Area under curve analysis and production of figures was performed using GraphPad Prism V.5.

3. **RESULTS**

3.1 IP test

The sham injection or handling treatments caused little perturbation in the plasma glucose concentration of yellowtail kingfish. Small fluctuations were observed between 1 and 3 hours following each of these procedures (Figure 1, Table 2), however, as the response was limited only the effect of intra-peritoneal injection of fish with D-glucose was evaluated in more detail.

Kingfish plasma glucose levels peaked at 12.8 ± 0.8 mM (mean \pm SEM) approximately 2 hours following injection of 1g D-glucose kg BW⁻¹ (Figure 1, Table 2). Baseline plasma glucose levels recorded at 0 hours were approximately 3.2 ± 0.1 mM. Circulating plasma glucose levels returned to baseline levels approximately 12 hours after injection and there was no statistical difference between circulating levels of plasma glucose at 0, 12, 18, 24, 48 or 72 hours (Table 2).

Total AUC of yellowtail kingfish injected with 1g D-glucose kg BW⁻¹ was significantly higher than in fish given either the sham injection or handling stress, however there was no difference in the total AUC of the procedural control treatments (Table 3). Similarly, Y_{max} was significantly higher in kingfish injected with glucose compared to both control treatments which were similar (Table 3). There was no affect of treatment on X_{max} (*P*>0.05) or X_{final} (*P*>0.05) (Table 3).

3.2 Oral test

Yellowtail kingfish given oral doses of 1, 3 or 6g D-glucose kg BW⁻¹ kingfish experienced peak plasma glucose concentrations of 10.8, 14.4 or 22.9 mM, respectively (Figure 2, Table 4). All treatments, excluding the fish fed the low CHO diet experienced peaks in plasma glucose concentration after 6 hours (Table 4). Similarly, regardless of dose, all kingfish were able to clear assimilated glucose approximately 18 hours after feeding; multiple comparisons procedures indicated plasma glucose concentrations were statistically similar at 0, 18, 24, 36 and 48 hours for all glucose treatments (Table 4). There was a significant but small elevation in plasma glucose following consumption of the low CHO control diet at the same relative feed intake as kingfish fed the 6g D-glucose kg BW⁻¹ treatment. This elevation was minor compared to peak glycaemic responses following ingestion of diets containing glucose (Table 4).

Total AUC and Y_{max} increased significantly (both *P*<0.05) in response to graded increases in the oral dose of D-glucose (Table 5). Glucose dose rate had no affect on X_{max} ,(*P*>0.05) however the X_{max} of fish fed the low CHO control diet was significantly lower than those fed 3 or 6g D-glucose kg BW⁻¹ (Table 5). Glucose dose rate had no affect on X_{final} (Table 5).

Glucose tolerance tests are a quick and effective method for gaining insight into the ability of fish to utilise CHO's. Two routes for the uptake of glucose were employed in the current study; intraperitoneal injection and oral intake. Regardless of the route of uptake, yellowtail kingfish exhibited prolonged hyperglycaemia for several hours. However, yellowtail kingfish were also able to clear circulating glucose from the blood stream relatively quickly and somewhat faster than has been observed in many other carnivorous teleosts (Anderson, 2003; Booth et al., 2006; Rowney et al,. 2008).

Limited research has been carried out on the ability of various *Seriola* species to utilise glucose. This is perhaps because they were previously regarded as intolerant of CHO's due to their strictly carnivorous nature. For example, earlier work with *S. quinqueradiata* fed 1.67g D-glucose kg BW⁻¹ demonstrated that blood glucose concentration peaked at 11.6 mM after 3 hrs and hyperglycaemia persisted after 5 hrs. However, post-prandial clearance time was not evaluated much beyond that point (Furuichi & Yone, 1981).The response of *S. quinqueradiata* in that study was also compared to that of omnivorous common carp *Cyprinus carpio* or semi-carnivorous red sea bream *Chrysophrys major*. Both those species were found to be far more efficient at clearing glucose from their bloodstream than *S. quinqueradiata*.

In contrast, yellowtail kingfish *S. lalandi* appear to assimilate and then clear an acute intraperitoneal challenge of glucose relatively quickly. For instance, similar studies to this one have been conducted with other aquaculture species using very similar experimental techniques. Three such 'carnivorous' examples challenged with an intra-peritoneal dose of 1 g D-glucose kgBW⁻¹ include mulloway, *Argyrosomus japonicus (Sciaenidae)* (Rowney et al., 2008), Australian snapper *Pagrus auratus* (Booth et al., 2006) and barramundi *Lates calcarifer* (Anderson, 2003). Plasma glucose concentration peaked at 6, 3 and 8 hrs, respectively for each of those species and the time taken to return plasma glucose concentrations to basal levels generally took longer than 18-24 hours. In contrast, yellowtail kingfish administered the same intra-peritoneal dose reached peak concentrations within an hour and clearance time within 12 hours (Figure 1). The rapid assimilation and clearance of glucose by kingfish was in fact similar to the response curves presented for the omnivorous silver perch *Bidyanus bidyanus* (Stone et al., 2003b) and tilapia *Oreochromis mossambicus* (Anderson, 2003), which both reached peak glucose levels after 1-2 hrs and returned to baseline concentrations within 6-12 hours (Figure 3).

Yellowtail kingfish fed increasing concentrations of glucose ranging from 1 to 6 g kgBW⁻¹ exhibited a systematic and incremental rise in peak glucose concentration, however, assimilation and clearance times were not dramatically different. The ability of fish to assimilate and clear increasing doses of glucose has been documented in several other species (Anderson, 2003; Gisbert et al., 2003; Stone et al., 2003b). Silver perch were injected intra-peritoneally with 2 or 4g Dglucose kg BW⁻¹. Unlike the systematic response seen in kingfish, silver perch injected with either dose displayed almost identical peak plasma glucose concentration, however fish injected with 4g D-glucose kg BW⁻¹ endured a longer period of hyperglycaemic (Stone et al. 2003b). Barramundi administered an intra-peritoneal dose of 4g D-glucose kg BW⁻¹ displayed a higher peak concentration than fish administered a 2g dose, but fish given the 4g dose experienced an extended hyperglycaemic state for well over 24 hours. The peak plasma glucose response observed in silver perch was suggested to be related to the flooding or overloading of the metabolic pathways related to glucose metabolism (Stone et al,. 2003b). Buddington (1987) reported that the capacity for intestinal glucose absorption in both the carnivorous rainbow trout Salmo gairdneri and the omnivorous common carp was not the limiting factor in glucose utilisation and even suggested that neither species would be able to consume enough glucose to saturate their respective intestinal absorptive capacity. Although the route of glucose administration differed between silver perch and yellowtail kingfish, the mechanisms constraining the uptake of glucose by silver perch (Stone et al. 2003b) do not appear to be operating in kingfish.

The fate of glucose following absorption into the blood can vary significantly. Once immediate energy needs of the fish are met, excess glucose may be stored in the liver and muscle tissue as glycogen (Wilson, 1994; De Silva & Anderson, 1995), removed from the body via excretion across the gills (Hemre & Kahrs, 1997) or the glycosuria pathway (Furuichi et al., 1986; Deng et al., 2001). The rapid assimilation and clearance of glucose by kingfish regardless of dose rate or route of administration suggest that the pathways and mechanisms involved in the removal of glucose from the plasma such as insulin and other glucose transport mechanisms are relatively efficient in kingfish. Alternatively, kingfish may have been able to up-regulate clearance mechanisms in response to blood glucose concentration, Rates of glycosuria have been found to increase significantly in both *S. quinqueradiata* (Furuichi et al., 1986) and tilapia (Lin et al., 2000) when challenged with increasing doses of glucose. The increase in rate of glycosuria was attributed to an overload of the renal glucose re-absorption threshold, where the majority of excess glucose that cannot be processed by the kidneys is excreted in the urine (Lin et al., 2000). Further examination of all these mechanisms will be necessary to gain a greater understanding of CHO metabolism in yellowtail kingfish.

Glucose turnover rates in fish have been found to be strongly influenced by metabolic rate. Utilisation and turnover in trout was found to increase substantially with increases in fish exercise and metabolic rate (West et al., 1993; Weber & Haman, 1996). The glucose turnover rate found in Skipjack tuna *Katsuwonus pelamis* has been likened to that of mammals and has been largely attributed to their high metabolic rate and energy demand (Weber et al., 1986). This may help partly explain the higher efficiency of glucose assimilation and clearance experienced in kingfish compared to the slower rates observed in the other carnivorous species we have examined. Yellowtail kingfish are an extremely active species and although they are well designed for swimming, they no doubt expend an enormous amount of energy during this process. Recent research has shown that yellowtail kingfish have a very high routine metabolic rate, comparable to other pelagic marine carnivores (Clark & Seymour, 2006). This rate is almost double the routine metabolic rate of mulloway (Pirozzi & Booth, 2009).

AUC analysis provides a measure of the glycaemic response of an animal to various foodstuffs and is used in the calculation of the glycaemic index of foodstuffs for humans and other mammals (Jenkins et al., 1981). AUC analysis is a widespread tool in the evaluation of total glucose and insulin levels in humans and mammals subjected to glucose tolerance tests, but its use in metabolic studies on fish is less prevalent (Deng et al., 2001). The low level glycaemic response of kingfish following either the sham or handling treatments in the IPTT trial are most likely due to an increase in endogenous glucose production which may be a response to stress or an increase in general activity during and following the treatment application process. In contrast, the small increase in the glycaemic response of kingfish fed the low CHO control diet is most likely due to increases in metabolic rate associated with post-prandial feeding responses (i.e specific dynamic action; SDA). Periods of stress have been found to significantly influence the endogenous production of glucose in fish and are often overlooked in glucose tolerance tests, often confounding the results. During the IPTT, handling and sham injection controls were included to account for methodological stressors involving in administration of glucose to fish. The fact that the levels of plasma glucose recorded in fish assigned to the control treatments was low and similar indicated that the use of anaesthetic to reduce the level of stress experienced by fish prior to the injection or handling procedures was effective. In addition, because all blood was obtained from unanaesthetised fish in less than 60 seconds, elevations in plasma glucose due to the collection procedure was avoided. The subdued response to the control diet in this study reflects the glycaemic response of yellowtail kingfish fed a research diet containing 100% pre-gelatinised wheat starch, indicating the availability and form of the CHO contained in the diet is also an extremely important factor governing the uptake and clearance of glucose from the blood system (see appendix).

5. CONCLUSION

Yellowtail kingfish *Seriola lalandi* administered an intra-peritoneal injection of 1g D-glucose kg BW⁻¹ experienced a peak plasma glucose concentration of 12.8 mM and hyperglycaemia for approximately 12 hours. When challenged with an oral dose of 1, 3 or 6 g D-glucose kg BW⁻¹, kingfish showed an incremental increase in peak response, however they continued to remove glucose from the bloodstream in a similar amount of time. Previously regarded as extremely intolerant of CHO, our results indicate that yellowtail kingfish may be able to utilise CHO more efficiently than once thought. This suggests they may tolerate increased levels of dietary CHO which could be extremely important for the development of new aquafeeds for this species.

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Ingredient composition of glucose and control test diets

Ingredient (g kg ⁻¹)	Glucose	Control
Peruvian fishmeal (Empresa pesquera)	237	397
Meat meal	90	150
Poultry meal	60	100
Fish oil	30	50
Dehulled lupin	120	200
Extruded wheat	60	-
Vitamin/mineral premix	3	3
D-glucose	400	-
High Protein maize gluten	-	100

Major ingredients supplied by Ridley AquaFeed Pty. Ltd, Narangba, Qld Australia.

Maize gluten supplied by Penfords Australia Pty Ltd. D-glucose provided by University of Technology Sydney (UTS)

TABLE 2

Plasma glucose concentrations (mM) of yellowtail kingfish following injection of 1g D-glucose kg BW⁻¹, a sham (saline) injection or handling control.

Time sampled (hr)	Glucose	Saline	Handled
0	2.7 . 0.03	2.5.4.0.23	2.5 ± 0.1^{3}
0	$3.7 \pm 0.0^{\circ}$	$3.5 \pm 0.2^{\circ}$	$3.5 \pm 0.1^{\circ}$
1	12.3 ± 0.8^{e}	4.5 ± 0.2^{bc}	4.2 ± 0.2^{b}
2	$12.8\pm0.8^{\text{e}}$	$3.3\pm0.3^{\circ}$	4.0 ± 0.3^{ab}
3	$10.4\pm0.3^{\text{d}}$	4.0 ± 0.4^{abc}	4.0 ± 0.1^{ab}
6	$7.5\pm0.8^{\circ}$	3.7 ± 0.3^{ab}	3.8 ± 0.1^{ab}
9	6.0 ± 1.1^{bc}	3.8 ± 0.2^{ab}	3.9 ± 0.2^{ab}
12	4.5 ± 0.7^{ab}	$3.5\pm0.2^{\rm a}$	3.9 ± 0.2^{ab}
18	3.9 ± 0.3^{a}	3.8 ± 0.2^{abc}	3.6 ± 0.1^{a}
24	4.0 ± 0.1^{a}	$3.6\pm0.2^{\rm a}$	$3.6\pm0.2^{\rm a}$
48	3.9 ± 0.1^{a}	3.9 ± 0.2^{abc}	3.6 ± 0.3^{ab}
72	3.7 ± 0.1^{a}	3.5 ± 0.3^{a}	3.7 ± 0.1^{ab}

Different superscript letters indicate significant row-wise differences between mean plasma glucose concentrations (oneway *ANOVA*). Values represent mean \pm SEM (n=4).

Total area under curve (AUC), peak glucose response (Y_{max}) , time of peak response (X_{max}) and clearance time (X_{final}) for yellowtail kingfish administered an intra-peritoneal injection of 1g D-glucose kg BW⁻¹, sham or handling control.

	Total AUC	Y _{max}	X _{max} *	X _{final}
	(mM hr)	(mM)	(hr)	(hr)
1g D-glucose kg BW ⁻¹	67.0 ± 4.3^{b}	13.6 ± 0.2^{b}	1.8 ± 0.3^{a}	38.8 ± 15.6^{a}
Sham injection	24.1 ± 6.4^{a}	4.5 ± 0.3^{a}	17.5 ± 10.9^{a}	35.8 ± 20.0^{a}
Handled	17.9 ± 2.0^{a}	4.4 ± 0.1^{a}	14.8 ± 11.2^{a}	25.6 ± 13.0^{a}

Different superscript letters indicate significant row-wise differences in each response variable. X_{max} data were log transformation to meet assumptions of *ANOVA*. Values represent mean ± SEM (n=3).

TABLE 4

Plasma glucose concentrations (mM) of yellowtail kingfish fed 1, 3 or 6g D-glucose kg BW^{-1} or a low CHO reference diet (control).

Time Sampled (hr)	1g glucose	3g glucose	6g glucose	Control
<u>^</u>	$2 2 + 0 1^{2}$	25.02		
0	$3.2 \pm 0.1^{\circ}$	$3.5 \pm 0.3^{\circ}$	$3.3 \pm 0.2^{\circ}$	3.2 ± 0.1^{400}
1	6.2 ± 1.0^{b}	5.8 ± 0.5^{b}	7.1 ± 0.6^{ab}	4.5 ± 0.8^{bcd}
2	6.6 ± 1.0^{b}	$8.3\pm0.6^{\rm c}$	9.9 ± 1.3^{bc}	5.0 ± 0.2^{d}
3	$8.4\pm0.4^{\text{c}}$	12.6 ± 0.2^{d}	10.5 ± 1.3^{bc}	4.9 ± 0.5^d
6	10.8 ± 0.7^{d}	14.4 ± 1.1^{d}	22.9 ± 2.8^{e}	4.4 ± 0.5^{abcd}
9	7.5 ± 1.1^{bc}	12.8 ± 1.1^{d}	15.2 ± 1.9^{d}	4.6 ± 0.2^{cd}
12	6.0 ± 0.4^{b}	$8.2\pm0.7^{\circ}$	14.2 ± 2.7^{cd}	4.0 ± 0.3^{abcd}
18	3.2 ± 0.0^{a}	4.2 ± 1.1^{ab}	7.2 ± 0.4^{ab}	4.0 ± 0.7^{abcd}
24	$3.5\pm0.4^{\rm a}$	$3.1\pm0.6^{\text{a}}$	4.7 ± 0.7^{a}	3.4 ± 0.2^{abc}
36	3.1 ± 0.2^{a}	$3.5\pm0.5^{\text{a}}$	$3.3\pm0.2^{\rm a}$	3.2 ± 0.1^{ab}
48	3.2 ± 0.2^{a}	3.6 ± 0.1^{a}	3.2 ± 0.3^{a}	3.1 ± 0.0^{a}

Different superscript letters indicate significant row-wise differences in mean plasma glucose concentrations (oneway ANOVA). Values represent mean \pm SEM (n=3).

Total area under curve (AUC), peak glucose response (Y_{max}), time of peak response (X_{max}) and clearance time (X_{Final}) for yellowtail kingfish fed 1, 3 or 6g D-glucose kg BW⁻¹ or a low CHO control diet.

	Total AUC* (mM hr)	Y _{max} * (mM)	X _{max} (hr)	X _{Final} (hr)
1g D-glucose kg BW ⁻¹	72.0 ± 4.1^{a}	$10.8\pm0.7^{\text{a}}$	6.0 ± 0.0^{ab}	24.6 ± 5.3^a
3g D-glucose kg BW ⁻¹	120.6 ± 11.2^{b}	14.5 ± 1.1^{b}	7.0 ± 1.0^{b}	20.8 ± 1.9^{a}
6g D-glucose kg BW ⁻¹	$208.6\pm27.8^{\rm c}$	$22.9\pm2.8^{\rm c}$	6.0 ± 0.0^{b}	41.5 ± 4.0^{a}
Control	25.0 ± 5.2^{d}	5.4 ± 0.4^{d}	3.0 ± 1.5^{a}	27.3 ± 10.7^{a}

*Raw data subjected to log transformation to meet ANOVA assumptions. Superscript letters indicate significant differences between means within each treatment . Values represent mean \pm SEM, (n=3).





Plasma glucose levels (mM) of yellowtail kingfish following an intra-peritoneal injection of 1g D-glucose kg BW⁻¹, sham injection or handling control (exp. 1). Values represent mean \pm SEM (n=4.)



Plasma glucose levels (mM) of yellowtail kingfish following an oral dose of 1, 3 or 6 g D-glucose kg BW⁻¹ or a low CHO diet (exp. 2). Values represent mean \pm SEM, (n=3).



Comparative response of yellowtail kingfish *Seriola lalandi*, mulloway *Argyrosomus japonicus*, barramundi *Lates calcarifer*, snapper *Pagrus auratus*, silver perch *Bidyanus bidyanus* and tilapia *Oreochromis mossambicus* to an intra-peritoneal injection of 1g D-glucose kg BW⁻¹.

4.15 Performance of yellowtail kingfish *Seriola lalandi* fed increasing dietary levels of extruded wheat or pre-gelatinised wheat starch

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ABSTRACT

This study examined the ability of juvenile yellowtail kingfish Seriola lalandi (mean stocking weigh 56.4g) to utilise increasing levels of two carbohydrate (CHO) sources; extruded wheat (EW) or pre-gelatinised wheat starch (PGN). A summit-dilution design was adopted whereby a high protein reference diet was systematically replaced (diluted) by EW, PGN or diatomaceous earth (DE) at 10, 20, 30 or 40%. Fish were reared at a temperature of 22°C under a 12L:12D photoperiod and fed restrictively, twice daily for a period of 28 days. Restrictive rations were maintained by adjusting rations following weekly weight checks. Fish fed diets containing diatomaceous earth lost weight as the experiment progressed indicating the limiting contribution of the reference diet to their nutritional requirements. Apart from fish fed diets containing DE and 40% PGN, the relative weight gain of kingfish remained high (17.9-24.3g kgBW^{-0.8}d⁻¹), even at elevated CHO inclusion content, with relative weight gain of kingfish fed the reference diet and those fed diets containing up to 40% EW or 30% PGN being similar. Feed conversion ratios also remained relatively stable in fish fed diets containing up to 40% EW and 30% PGN (i.e. 1.3-1.6), however FCR got progressively worse in fish fed diets substituted with increasing levels of DE. Yellowtail kingfish recorded protein efficiency ratios (PER) of approximately 1.6 when fed diets containing EW or 1.7 when fed diets containing PGN, regardless of ingredient inclusion level, indicating that protein sparing was occurring. Hepatosomatic index of kingfish fed both CHO sources remained unchanged or lower than values recorded in fish fed the reference diet. Relative gut length (RGL) of yellowtail kingfish fed the reference or CHO diets was not different at the conclusion of the trial with RGL values of approximately 0.6 recorded in all fish. The efficient utilisation of EW and PGN by juvenile yellowtail kingfish reared under the conditions imposed by this experiment indicates that increased levels of dietary CHO and moderate reductions in dietary protein are possible without overly affecting growth performance and protein retention.

1. INTRODUCTION

Carbohydrates (CHO) can be an inexpensive source of energy and are vital as a binding agent in compound aquaculture feeds. However strong inter-specific differences in CHO utilisation and a lack of understanding regarding the metabolism of dietary CHO by fish has meant their adoption as an energy source in aquafeeds remains problematic (Wilson, 1994; Legate, et al., 2001; Moon, 2001; Hemre et al., 2002). The relative inability of fish to physically digest and absorb dietary CHO as well as their apparent inability to efficiently metabolise glucose has been the focus of much research. Most unrefined dietary CHO sources contain a mixture of non-starch structural polysaccharides (NSP's) as well as energy-reserve polysaccharides (starches) (Stone et al., 2003; Krogdahl et al., 2005). NSP's such as cellulose are generally indigestible by most fish (Krogdahl et al., 2005) whilst starch is composed primarily of α -amylase which is formed by the bonding of glucose residues and therefore forms a considerable potential energy source (De Silva & Anderson 1995). In fish, digestibility of CHO appears to be dependent on the complexity of the CHO source as well as inclusion level in the diet, with simpler CHO's being more easily digested while a general reduction in digestion efficiency occurs as dietary inclusion content increases (Hemre et al., 1989; Krogdahl et al., 2005; Booth et al., 2006).

In general there is a strong relationship between the natural trophic level of a species and its ability to utilise CHO as an energy source, with herbivorous and omnivorous species showing much

greater potential than carnivorous species (Wilson 1994; De Silva & Anderson 1995; Legate et al. 2001; Moon et al. 2001; Hemre et al. 2002). For example, omnivorous species such as silver perch (*Bidyanus bidyanus*) are able to efficiently utilise up to 30% dietary starch (Stone et al. 2003), but dietary starch levels of as little as 10% have been shown to have immediate detrimental impacts on feed utilisation in carnivorous Atlantic Salmon (*Salmo salar*) (Hemre et al. 1995, cited in Hemre et al. 2002).

Processing of CHO sources such as extrusion or gelatinisation has been found to increase digestibility of CHO's in most fish species, largely by breaking down the strong molecular structure of starch. However, relying primarily on digestibility information in formulating diets is risky. Furuichi et al. (1986) found that Japanese amberjack (*Seriola quinqueradiata*) fed potato starch consistently outperformed fish fed glucose at a 20% dietary inclusion level despite the significantly higher digestibility of the glucose. This is most likely due to inefficiency in the utilisation of glucose once in the bloodstream; post prandial glucose levels were significantly higher in fish fed glucose than the potato starch and indicated that a slower release of glucose into the blood may be beneficial for growth, as hyperglycaemia and excess glucose excretion can be avoided.

CHO's are used in modern aquafeeds as a binding agent in the pelleting process and where applicable as a dietary energy source. They are generally a cheaper form of dietary energy than other energy sources such as protein and lipids. As indicated, the dietary inclusion level of CHO will depend on species, CHO complexity and state of gelatinisation, digestibility and inclusion level. There may also be interactions between CHO sources or other ingredients to consider. In general, the level of CHO is low in the diets of carnivorous species and often is less than about 15%. Most omnivorous and herbivorous species will tolerate much higher levels.

Apart from their physical ability as binding agents, CHO's can be used as an alternative energy source capable of sparing dietary protein (amino acids) primarily for protein synthesis (De Silva & Anderson 1995). For some carnivorous species, especially salmonids, dietary lipid has been the preferred source of exogenous energy for sparing protein for growth. However, some marine fish do not utilise high dietary lipid contents and marine lipids such as fish oil are becoming increasingly expensive. There is therefore a reason to optimise the use of CHO's to satisfy or contribute to metabolic energy demands (Erfanullah & Jafri 1995; Stone et al 2003; Wu et al 2007). Although the sparing effect of CHO sources have been shown in numerous studies (Erfanullah & Jafri 1995; Hemre & Hansen 1998; Shiau & Lin 2001; Peres & Olivia-Teles 2002; Stone et al 2003; Wu et al 2007), the preferential use of amino acids over glucose for catabolic purposes means its success in diets relies heavily on the correct balance of dietary protein and energy.

Yellowtail kingfish (YTK) (*Seriola lalandi*) are a pelagic marine carnivore and an exciting new prospect for Australian aquaculture. Highly prized as a sashimi fish, YTK are seen as having huge aquaculture potential for both domestic and export markets (Love & Langenkamp 2003). Currently, Australian YTK diets contain between 10-15% CHO which is mostly incorporated for its binding properties. These low levels are also based on earlier research with other *Seriola* species such as the Japanese Amberjack (*Seriola quinqueradiata*) and Greater Amberjack (*Seriola dumerili*) that indicated CHO's were poorly utilised by these active teleosts. Little if any nutritional research has been carried out on the CHO tolerance of yellowtail kingfish and its ability to use exogenous dietary CHO's to satisfy metabolic energy demands.

The aim of this experiment was to evaluate the ability of juvenile YTK to utilise increasing dietary levels of two carbohydrates sources; extruded wheat (EW) or 100% pre-gelatinised wheat starch (PGN). Dietary levels of each CHO were increased by employing a summit / dilution approach whereby a high protein reference or summit diet was replaced by 10, 20, 30 or 40% by weight of EW or PGN. Diatomaceous earth (DE; inert filler) was used as a control to replace similar quantities of the reference diet. The effect of each ingredient was evaluated by monitoring changes in weight gain, feed efficiency and protein efficiency ratio. Condition factor, hepatosomatic index and adaptive modification of gut length were also examined to assess the effect of CHO inclusion

level on general organ health and physical condition. Differences in these indices for fish fed diets containing EW or PGN compared with those fed DE were interpreted as indicative of the nutrient contribution of EW or PGN.

2. MATERIALS AND METHODS

2.1 Experimental fish and facilities

This study used juvenile yellowtail kingfish (*Seriola lalandi*) (mean weight 56.4g, n=576) which were progeny of wild-caught broodstock held at the Port Stephens Fisheries Institute, NSW, Australia. Prior to the experiment all fish were housed in a 10kL tank and fed a commercial finfish diet; 50% CP, 12% fat (Ridley AquaFeed Pty. Ltd, Narangba, Qld Australia).

The experiment was performed in a laboratory that housed 48 x 200L white polyethylene aquaria supplied by two interlinked recirculating bio-filtration units of 1,700 litre capacity. Water flow to each experimental tank was controlled by small PVC taps that provided a weak centripetal current within each tank that assisted the removal of waste through a central, vertical PVC standpipe (32mm diameter) fixed approximately 5mm from the base. Water temperature was controlled in each of the bio-filtration units using an immersion heater operating antagonistically against a chiller unit to allow precise temperature control ($22\pm1.0^{\circ}$ C). Each unit was enriched with medical grade oxygen which was injected directly into each of the main supply manifolds. Each of the 48 experimental aquaria contained a central air-stone to provide additional aeration. Black plastic was wrapped around each of the 48 aquaria and a black plastic lid, which covered half of the tank opening, was fitted to minimise external disturbance of fish. Black 'bird-mesh' was installed over each experiment tank to prevent fish escaping.

2.2 Experimental design

Increased feed intake has been found to occur in many fish in response to high levels of carbohydrates in diets or to account for nutritional inadequacies (Hemre et al. 2002; Peres & Oliva-Teles 2002). To accurately measure the utilisation of each test ingredient and the inert filler (diatomaceous earth), a restricted feeding regime was implemented to ensure fish could not increase feed intake to overcome nutritional deficiencies. The experimental design included a restricted feeding regime for all fish fed on summit / diluent diets (i.e. 13 treatments). To assess the impact of the restricted feeding regime another group of fish were fed the summit reference diet to apparent satiation throughout the experiment. Two other treatments were established to assess the effect of weekly weight check procedures on kingfish. These treatments were fed on a commercial feed to apparent satiation twice per day. One group were weighed each week as per other treatments and the other group was not handled during the trial (i.e. total of 16 treatments). After stocking, 3 replicate tanks were randomly assigned to each of the experimental treatments (n=3).

2.3 Experimental diets

The ingredients used in the summit or diluent diets and their respective nutrient or energy contents are presented in Table 1. The formulations for each of the experimental diets and the their measured nutrient or energy contents are presented in Table 2, along with the measured nutrient or energy content of the commercial diet (Ridley 50/12 Marine Float; Ridley AquaFeed Pty. Ltd, Narangba, Qld Australia)

The summit diet was formulated to be high in protein and contained a mix of protein sources including fishmeal, poultry meal and dehulled lupins. It also contained extruded wheat (Table 2). A standard amount of extruded wheat was included in the summit diet as most extruded commercial feeds for yellowtail kingfish contain at least 10% wheat as the primary binding agent (R. Smullen; Ridley Aquafeed Pty Ltd, *pers. comm.*). Each of the experimental diets was then prepared by replacing 10, 20, 30 or 40% of the summit diet (by weight) with a similar amount of PGN, EW or
DE. (Table 2). A constant amount of 0.3% vitamin / mineral premix (DSM Nutritional Products, Wagga Wagga, NSW, Australia) was added to all diets except the commercial feed to ensure dilution of these micro-nutrients did not confound results.

Prior to manufacturing experimental feeds all raw ingredients were ground through a 1.5mm screen in a laboratory hammer mill in order to standardise ingredient particle size (C-E Raymond Inc. IL, USA). The commercial feed was also ground and remade to ensure the physical qualities of this diet were similar to other experimental feeds. Each diet was thoroughly dry mixed in a Hobart mixer (Hobart Corporation, Troy, OH, USA) before wet ingredients (i.e. fish oil and water) were added. The mash was formed into dough and divided equally. The first half of the mash was cold pressed through a 5mm die plate and the remaining half passed through a 6mm die plate fitted to a small scale meat mincer (Barnco Australia Pty. Ltd., Leichardt, NSW, Australia). Different pellets diameters were manufactured in order to accommodate the expected growth of the fish. After pelleting, all diets were dried at low temperature in a convection drier at <35°C for approximately 7 hours. Dry weight analysis carried out on all diets ensured uniform moisture contents of < 5%. Prior to and during the study all diets were frozen at <-15°C.

2.4 Feeding protocols

During the first week all fish were hand fed to apparent satiation twice daily (0900 & 1400h) in order to establish normal satiated feed intake levels for each treatment. Fish were then lightly anaesthetised in their respective tanks (10 mg Aqui-S L⁻¹) and bulk weighed (excluding non-handled treatment). The resultant weight gain and feed intake from the 1st week was then used to calculate the relative daily feed intake of each tank. This value was approximately 6% BWd⁻¹ so rations for week 2 were nominally restricted to 5%BWd⁻¹. Similar procedures were undertaken at the end of week 2 and week 3 whereby intake was futher restricted to 4.5 and 4%BWd⁻¹, respectively. The experiment was run for 4 weeks (28 days). At the conclusion of the experiment fish were anaesthetised (10 mg Aqui-S L⁻¹) and individual weight and fork length remeasured and recorded. In addition, 3 fish from each tank were euthanaised via an overdose of anaesthetic (50 mg Aqui-S L⁻¹) and frozen. These fish were later dissected to determine hepatosomatic index (HSI) and relative gut length (RGL). RGL was determined by measuring the intestinal length (anterior end of small intestine to vent) after gently extending the dissected intestine without stretching.

During the stocking process a larger population of fish were lightly anaesthetised in a 10kL holding tank (15 mg Aqui-S L⁻¹; active ingredient 50% iso-eugenol. Aqui-S New Zealand Ltd, Lower Hutt N.Z), captured at random and transferred into a 200L tank containing a stronger dose of anaesthetic (25 mg Aqui-S L⁻¹). These fish were then screened to ensure they were within the desired weight range before their individual weight and fork length was recorded. Fish were then distributed systematically to each of the 48 experiment tanks in groups of six until each experiment tank contained 12 fish. Initial stocking density was maintained throughout the experiment by replacing dead or moribund fish with fish of similar size. Replaced fish had their right pectoral fin clipped and were identified at harvest and excluded from final analyses.

2.5 *Performance indices*

Relative weight gain (RWG), feed conversion ratio (FCR), protein efficiency ratio (PER), hepatosomatic index (HSI), relative gut length (RGL) and condition factor (CF) were calculated for each experimental treatment using the following equations:

Relative weight gain (RWG) (g kg^{-0.8}d⁻¹) = [individual weight gain / ((geometric mean body weight / 1000)^{0.8}) / days].

Feed conversion ratio (FCR) = dry weight feed consumed / wet weight gain of fish

Protein efficiency ratio (PER) = daily weight gain / daily crude protein intake]

Hepatosomatic index (HSI%) = wet weight of liver / whole body weight x 100

Relative gut length (RGL) = intestinal length / fork length of fish

Fulton's condition factor (CF) = (weight (g) x 100000) / (fork length (mm³))

2.6 Water quality

During the feeding trial, temperature $(22^{\circ}C \pm 1^{\circ}C)$, pH (Range 7.08-7.76), salinity (27.3-32.5 ppt) and dissolved oxygen (>6 mg L⁻¹) were measured daily using a Horiba U10 water quality analyser (Horiba, Japan). Total ammonia was recorded daily (<0.6mg L⁻¹) using a rapid colourmetric method (E.Merck, Model 1.08024, Germany). For the duration of the trial, photoperiod was controlled with fluorescent lighting to mimic ambient 12 L:12 D conditions.

2.7 *Chemical analyses*

All test ingredients and diets were analysed for crude protein, crude lipid, gross energy, ash and dry matter according to Association of Official Analytical Chemists (AOAC 1995). Crude protein (Nx6.25) was determined according to the Dumas Method. Total lipid was determined using ether extraction. Gross energy (MJ kg⁻¹) was determined using bomb calorimetry. Ash was determined gravimetrically following HCI wash after incineration at 550°C for 2 hrs. Dry matter was measured gravimetrically after drying in an oven at 105°C for 16hrs. Analysis of all ingredients and diets was performed by QDPI Health & Nutritional Biochemistry Unit (Yeerongpilly, Qld, Australia).

2.8 Statistical analysis

Two-way analysis of variance (*ANOVA*) was used to examine the interactive effects of ingredient type (EW, PGN or DE) and inclusion level (10, 20, 30 or 40%) (both treated as fixed factors), on relative weight gain (RWG), feed conversion ratio (FCR), protein efficiency ratio (PER), hepatosomatic index (HSI), relative gut length (RGL) and condition factor (CF). The average value for each of these indices (expressed as the mean of 12 fish from each tank) was used as 1 of 3 replicate data points in the statistical procedures. A one-way *ANOVA* was used to compare the performance indices of all treatments including non-handled and handled kingfish fed the commercial diet. Prior to statistical analysis, all raw data were examined for homogeneity of variances (Cochran's C test). Where necessary, data were log transformed to ensure this assumption was satisfied. Where significant differences were found between treatment means, Tukeys post-hoc HSD multiple comparisons procedure was carried out to separate the means. All analyses were performed using Statgraphics Plus 4.1 for Windows (Manugistics, Inc.Rockville, Maryland, U.S.A.) at the 95% confidence interval. Figures were produced using Microsoft Excel (Microsoft Corporation, USA).

3. **RESULTS**

All fish survived with the exception of kingfish allocated to the 40% DE diet. Fish on this treatment experienced 22% mortality during the final week of the trial. Mortality in this treatment was not confined to any particular replicate and was most probably related to nutritional deficiency due to the restricted feeding regime. All fish from this treatment were emaciated at the end of the experiment but showed no other clinical signs of disease or illness. Palatability of feeds containing DE was not an issue, as all fish in this series displayed ravenous feeding behaviour and no rejection of ingested pellets. Performance indices recorded for each treatment at the end of the trial are presented in Table 3 while organ and condition indices are presented in Table 4.

Two-way ANOVA indicated that RWG, FCR and PER were all significantly affected by the main effects (P < 0.01) and the interaction of those terms (P < 0.01). The highly significant interaction term

in regards to all performance indices is due to the significant and systematic reduction in body weight of fish reared on diets substituted with DE, especially those at the extreme end of the series, compared with a different response for fish fed EW or PGN. The response of yellowtail kingfish fed on the DE diet series was dramatically different to that observed for fish fed the EW or PGN series of diets (Table 3; Figure 1) and confirms the limiting contribution of the summit diet in meeting the nutritional requirements of these rapidly growing fish under our experimental regime. Similarly, two-way ANOVA indicated that HSI and RGL were significantly affected by each of the main effects (P<0.05) and their interaction (P<0.05). In contrast, CF was significantly affected by each of the main effects, but not the interaction of terms ($F_{2,24}$ =1.9, P>0.05).

Performance data subjected to one-way *ANOVA* are presented in Table 3 and Table 4. These analyses indicate that there was no significant difference between the RWG, FCR or PER of kingfish fed slightly restricted rations compared to fish fed to apparent satiation (i.e. reference restricted vs reference satiated). In addition, the similarity in the weight gain, FCR or PER of yellowtail kingfish not handled or subjected to repetitive weekly handling procedures (i.e. commercial diet treatments) indicates that the weighing procedure did not influence the performance of fish in this study. However, kingfish fed on the commercial barramundi diet recorded significantly lower weight gain, poorer FCR and PER than fish fed the summit diet or in fact many of the other diets, which indicates this diet was not optimal for this species.

RWG, FCR and PER in fish fed on the EW diets and the summit diet were statistically similar. This was not the case for the PGN series, where RWG and FCR of fish receiving the diet containing 40% PGN were statistically different to the summit diet (Table 3). However, PER was remarkably stable and was little affected by the increasing concentration of either CHO ingredient (Figure 2).

The response of PER to increasing dietary crude protein intake was examined for each diet series (Figure 3). This examination revealed that PER of fish fed the EW or PGN series of diets was relatively unaffected by increasing protein intake (i.e. parallel to x-axis). In contrast, the PER of fish fed the DE series improved as protein intake increased. This clearly demonstrated that fish fed the diets containing DE, particularly the diets containing very high levels of DE were catabolising protein intake implied these ingredients were sparing protein for growth.

4. DISCUSSION

Dramatic reductions in the weight gain and PER of fish subjected to the DE series clearly demonstrated the declining contribution of the summit diet in supporting normal weight gain and growth. The absolute amount of protein and energy decreased in this series as the level of DE increased, however the ratio of protein:energy was maintained. In contrast, the weight gain and PER of fish reared on diets containing increasing levels of EW or PGN was sustained and relatively stable for all but the most extreme treatments. As both EW and PGN contain low or no protein, respectively, the sustained performance of kingfish reared on the EW and PGN series of diets must be due to a significant protein sparing effect of EW or PGN for protein in the summit diet.

Despite the inability of the majority of teleosts to efficiently process glucose when compared with mammals, many species of fish have been found to efficiently utilise dietary CHO for energy when it is included in the diet at low levels (Erfanullah 1995; Hemre et al. 2002; Stone et al. 2005; Wu et al. 2007). Yellowtail kingfish as they were able to maintain relatively high growth rates even at the extreme inclusion of both EW and PGN. In comparison, *S. quinqerdiata* grown at comparable water temperatures and fed graded levels of potato starch (Furuichi et al. 1986) grew at half the rate of the yellowtail kingfish in the present study (*S. quinqerdiata* fed 10 or 20% potato starch exhibited RWG of approximately 14.4 and14.02 g kg^{-0.8}d⁻¹, respectively). In contrast, yellowtail kingfish from the present study recorded RWG of 22.9 and 20.9 g kg^{-0.8}d⁻¹ when fed 10 or 20% PGN, respectively. This difference in growth may be due to inter-specific differences in their ability to utilise starch or differences in the digestibility of potato starch and PGN. Comparison of these experiments needs to

be made cautiously as there are also differences in methodology and the nutritional quality of the basal diets used in each study.

Many carnivorous fish species have been found to increase feed intake to maintain growth in response to increased amounts of starch-derived energy provided by high levels of CHO in the diet (Hemre 2002; Wu et al. 2007). Our adoption of a restricted feeding regime prevented yellowtail kingfish increasing feed intake to offset nutritional deficiencies. In this way, the most immediate nutritional issue for kingfish would be the systematic reduction in protein content (amino acid) as the CHO's were increased. The impact was more acute in the PGN series due to the lack of protein in this refined ingredient. This effectively forced kingfish to use the energy derived from the metabolism of CHO in order to channel protein into meeting their genetically programmed growth potential. Although not seen in the present study, carnivorous species have also shown increased feed efficiency on diets containing low levels of CHO compared to carbohydrate free diets (Hemre 2002; Wu et al. 2007). We observed a minor numerical increase in weight gain of fish fed the 10% EW diet, but because our fish were fed restrictively the influence of altered consumption in response to nutritional deficiencies could not be quantified. Therefore the small numerical increases (i.e. worsening) observed in FCR as either EW or PGN level increased were most likely due to the concomitant but small reductions in weight gain.

Many species that rely solely on dietary protein must budget amino acids for growth (anabolism) and energy needs (catabolism) in order to meet their growth or reproductive potential. Thus, if CHO is to be useful as an energy source in aquafeeds, fish must have the ability to utilise the energy from the CHO for catabolic purposes whilst preserving the maximum amount of protein (amino acids) for growth (i.e. protein synthesis) (Wilson 1994; De Silva & Anderson 1995). This ability is commonly referred to as 'protein sparing' and is also relevant to the use of lipids. The determination of protein efficiency ratio (PER) in experiments is useful in providing an insight into the effects of dietary protein and energy level (De Silva & Anderson 1995). In the present study, the PER observed in fish fed diets containing EW or PGN remained relatively stable and was even slightly higher than the PER of fish reared on the summit diet. Thus the relative balance and availability of protein and energy from these diets was apparently sufficient to maintain normal rates of protein synthesis under a restricted feeding regime. The subsequent small numerical reductions in growth as each of the CHO sources was increased were therefore related to reductions in dietary protein content and or absolute protein intake, a consequence of restricted feeding and the dilution of protein by each of the CHO sources. Increased retention of protein often results when fish are fed diets where protein intake is restricted slightly. However, when protein becomes limiting and fish are forced to catabolise amino acids for energy, protein retention falls dramatically. This effect was clearly displayed by kingfish fed the DE series of diets due to the concomitant reduction in protein and energy intake as inclusion of DE increased. The protein sparing effect of CHO's has been demonstrated in many species such as yellowfin bream Sparus latus (Wu et al. 2007), European seabass Dicentrarchus labrax (Peres & Olivia-Teles 2002) and freshwater catfish Mystus montanus (Raj et al. 2008).

Of major interest in this study was the similarity in relative weight gain of kingfish fed 10, 20 or 30% EW or PGN. This suggested each of these CHO sources was being equally well utilised. Extruded wheat contained 17% crude protein, 19 MJ gross energy kg⁻¹ and 75.7% NFE on a dry matter basis while PGN contained virtually no protein (0.6%), 17 MJ gross energy kg⁻¹ and was 98% NFE (Table 1). Thus on a gross nutrient or energy basis EW would appear to be the more appealing ingredient. The additional protein may in fact explain the slight numerical increase in the RWG of fish fed the diet containing 10% EW. However, the digestibility of the ingredients or diets used in this study was not quantified and differences in digestibility of the CHO sources could also explain the similarities in performance. For example the amount of digestible energy available from PGN may be higher than for EW while at the same time fish fed on the EW series of diets were benefiting from the additional digestible protein contributed by the EW. Differences in each of the wheat products were also apparent. The EW had been cooked in an extruder, however the level of gelatinisation was unknown while the PGN was 100% gelatinised and highly refined. On face value

it would appear the available energy from each of these products was fairly similar. The ability of fish to utilise CHO sources following processing such as cooking or extrusion has been well described and has generally been found to substantially increase their digestibility and subsequent utilisation (Wilson 1994; De Silva & Anderson 1995; Krogdahl et al. 2005).

Increased liver size as a result of glycogenesis or lipogenesis is a common side-effect of an oversupply of CHO derived energy in fish diets (Wilson 1994; Hemre et al. 2002; Peres & Olivia-Teles 2002; Booth et al. 2006). No major differences were found in the HSI of kingfish fed EW or PGN diets compared to the summit diet which is similar to results observed in several other species fed graded levels of different CHO sources (Hillestad et al. 2001; Wu et al. 2007; Sa et al. 2007; Rawles et al. 2008). This may indicate kingfish are able to efficiently regulate absorbed glucose without the need to upregulate glycogenesis or lipogenesis. Results from an exploratory pilot study (see appendix) indicate yellowtail kingfish showed no significant increase in glycaemic state when fed a diet containing 40% PGN compared to fish fed a low CHO control diet, but a significant and strong glycaemic response when fed a diet containing 40% D-glucose. This result was explained by the regulated digestibility and absorption of the PGN due to the need to hydrolyse the starch during digestion. This process ultimately slows absorption and facilitates a prolonged and therefore more utilisable release of glucose into the bloodstream. A similar process is likely operating for digestion of EW. This effectively reduces the metabolic costs of storing or excreting unwanted glucose, reducing energy demands.

Increasing intestinal surface area or increased gut length has been found to aid the digestion of CHO's sources in fish by potentially increasing intestinal absorptive capacity and prolonging gut clearance time (De Silva & Anderson 1995). Adaptive gut morphology is most commonly seen in omnivorous species which naturally rely on mixed sources of food as opposed to strictly carnivorous species which rarely consume non-protein foods (Stroband 1977; Buddington 1987; Yang et al. 2002; German & Hall 2006). We did not record any changes in relative gut length of yellowtail kingfish in the current study, however the length of our trial may have precluded the observation of this phenomenon. Adaptive changes in gut length in response to increased dietary CHO have been observed in other carnivorous species such as rainbow trout Oncorhynchus mykiss (Buddington 1987). Other physiological effects of dietary CHO in diets for fish have also been documented and include increased lipid levels and decreased liver function (Wilson 1994; Hemre et al. 2002; Wu et al. 2007). While these responses were not tested in the current study, there is enough evidence in the literature to indicate they are likely to be impacted by the inclusion of high levels of CHO. Therefore, although the results of our trial are encouraging, longer term studies should be undertaken to establish a true indication of the potential impacts of high CHO on organ and gut health as well as other important physiological mechanisms.

The physiological response and impacts on growth from acute and repeated stressors such as crowding, water quality, regular handling and even the use of anaesthetics have been well described (Rotllant & Tort 1997; Pottinger & Carrick 1999; Ortugo et al. 2002; Morales et al. 2005; Moran et al. 2008; Pirozzi et al. 2008) and have been found to detrimentally affect the scope for growth in many fish species. We recorded similar growth and feed efficiency in kingfish subjected to a repetitive handling stress as we did for fish that were not handled during the experiment. This demonstrated that the juvenile yellowtail kingfish can be sampled at regular intervals without these procedures affecting their performance. This will give researchers greater confidence in planning and operating experiments and that the results of these experiments will not be confounded by regular or interim weight checks on these animals.

5. CONCLUSIONS

Juvenile yellowtail kingfish *Seriola lalandi* were able to efficiently utilise both extruded wheat and pre-gelatinised wheat starch as energy sources. The fact that juvenile kingfish were able to maintain a relatively stable PER over nearly the entire range of CHO inclusion levels we investigated has demonstrated a strong 'protein sparing' effect by these ingredients. Despite a slight linear reduction

-n performance as inclusion of both ingredients increased, growth of kingfish fed EW up to 40% and PGN up to 30% were not significantly different from fish fed the reference diet. Analysis of HSI, RGL and CF indicated that the inclusion of high CHO in diets had minimal detrimental effect on internal organs or physical health. Results from this study indicate that, when compared to many other species of fish, and particularly those of a carnivorous nature, kingfish are able to efficiently utilise CHO for energy and that a reduction in dietary protein in aquafeeds for this species may be possible without compromising overall performance.

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TABLE 1

Analysed nutrient or energy composition of ingredients used to formulate test diets used in the experiment (dry matter basis)

Ingredient ratio)	Crude protein (%)	Crude lipid (%)	Total energy (MJ kg ⁻¹)	Ash (%)	NFE* (%)
Fishmeal (Grupo Sipesa)	72.5	8.6	20.4	17.6	1.3
Meat meal	50.0	10.0	15.0	39.5	0.5
Poultry meal	69.9	17.1	23.4	11.7	1.3
Dehulled lupin	42.8	7.3	20.8	2.6	47.3
Fish oil	0.0	98.0	39.3	0.0	0.0
Extruded wheat	17.0	4.6	19.1	2.7	75.7
Pregelled wheat starch	0.6	0.9	17.3	0.2	98.3
Diatomaceous earth	0.0	0.0	0.0	100	0.0

*Nitrogen-free extract calculated by difference [NFE % = 100 % - (crude protein % + crude fat % + ash %)] Note: Major ingredients supplied by Ridley AquaFeed Pty. Ltd, Narangba, Qld Australia. Pregelatinised supplied by Penfords Australia Pty Ltd.

Ingredient composition ⁻¹	Ref (100/0)	EW (90/10)	EW (80/20)	EW (70/30)	EW (60/40)	PGN (90/10)	PGN (80/20)	PGN (70/30)	PGN (60/40)	DE (90/10)	DE (80/20)	DE (70/30)	DE (60/40)	COMM**
Fishmeal (Grupo Sipesa)	397	357	318	278	238	357	318	278	238	357	318	278	238	-
Meat meal	150	135	120	105	90	135	120	105	90	135	120	105	90	-
Poultry meal	100	90	80	70	60	90	80	70	60	90	80	70	60	-
Extruded wheat	100	90	80	70	60	90	80	70	60	90	80	70	60	-
Dehulled lupin	200	180	160	140	120	180	160	140	120	180	160	140	120	-
Fish oil	50	45	40	35	30	45	40	35	30	45	40	35	30	-
Vitamin/mineral premix	3	3	3	3	3	3	3	3	3	3	3	3	3	-
Extruded wheat	-	100	200	300	400	-	-	-	-	-	-	-	-	-
Pregelled wheat starch	-	-	-	-	-	100	200	300	400	-	-	-	-	-
Diatomaceous earth	-	-	-	-	-	-	-	-	-	100	200	300	400	-
Nutrient composition														
Crude protein (%)	54.6	50.6	47.1	43.6	39.8	49	42.5	37.6	30.9	48.4	42.1	37.9	33.8	56.5
Crude lipid (%)	14.0	13	12.1	11.3	10.1	12.1	10.3	8.7	6.5	11.9	10.9	9.1	8.2	12.9
Gross energy (MJ kg ⁻¹)	20.8	20.7	20.7	19.9	19.9	20	19.5	19.4	18.9	18.6	16.4	14.4	12.6	22.1
Ash (%)	14.2	13.4	12.5	11.1	10.3	13.8	11.8	10.7	8.3	24.1	34	40.6	53	8.6
NFE* (%)	17.2	23	28.3	34	39.8	25.1	35.4	43	54.3	15.7	13	12.4	11.34	22

TABLE 2	Ingredient and nutrient co	mposition of test	diets (g kg ⁻¹	or MJ kg ⁻¹)
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*Nitrogen-free extract calculated by difference [NFE % = 100 % - (crude protein % + crude fat % + ash %)] **Ingredient composition of commercial diet unknown.

TABLE 3

Relative weight gain (RWG), food conversion ratio (FCR) and protein efficiency ratio (PER) calculated for fish at the conclusion of the 28 day growth trial. (Values represent mean \pm SEM of 3 experimental aquaria). Different superscript letters indicate significant differences between treatments within each performance indice (Tukeys HSD comparison). FCR data log transformed.

		DUIC	ECD	DED
Diet / Treatment	Basal / Ingredient	RWG	FCR	PER
PSFI reference restricted	100/0	$22.6\pm1.41^{\rm fg}$	1.3 ± 0.07^{a}	$1.5\pm0.09^{\text{defg}}$
PSFI reference satiated	100/0	$21.6\pm0.06^{\text{fg}}$	1.4 ± 0.05^{a}	1.4 ± 0.05^{de}
Extruded wheat	90/10	$24.3\pm1.12^{\text{g}}$	1.3 ± 0.07^{a}	$1.6\pm0.09^{\text{efg}}$
Extruded wheat	80/20	20.6 ± 1.70^{fg}	1.4 ± 0.10^{a}	$1.6\pm0.11^{\text{efg}}$
Extruded wheat	70/30	18.9 ± 1.07^{ef}	1.5 ± 0.08^{ab}	$1.5\pm0.08^{\text{efg}}$
Extruded wheat	60/40	17.9 ± 0.07^{cdef}	1.6 ± 0.02^{ab}	$1.6\pm0.02^{\text{efg}}$
Pre-gelatinised starch	90/10	$22.5\pm1.07^{\mathrm{fg}}$	1.3 ± 0.05^{a}	$1.6\pm0.07^{\text{efg}}$
Pre-gelatinised starch	80/20	20.9 ± 0.68^{fg}	1.4 ± 0.03^{a}	$1.7\pm0.04^{\rm fg}$
Pre-gelatinised starch	70/30	18.7 ± 0.11^{cdef}	1.5 ± 0.02^{ab}	$1.7\pm0.03^{\rm g}$
Pre-gelatinised starch	60/40	$14.0\pm0.75^{\text{cde}}$	2.0 ± 0.09^{bc}	$1.7\pm0.08^{\text{efg}}$
Diatomaceous earth	90/10	18.7 ± 0.34^{def}	1.5 ± 0.03^{ab}	$1.4\pm0.03^{\text{cde}}$
Diatomaceous earth	80/20	13.5 ± 0.28^{cd}	$2.1\pm0.03^{\text{c}}$	$1.1\pm0.02^{\text{cd}}$
Diatomaceous earth	70/30	7.0 ± 0.76^{b}	3.9 ± 0.31^d	0.7 ± 0.06^{b}
Diatomaceous earth	60/40	-0.1 ± 1.07^{a}	-13.2 ± 14.15^{e}	0.0 ± 0.11^{a}
Commercial handled	-	$13.0 \pm 1.15^{\circ}$	$2.5\pm0.20^{\rm c}$	0.7 ± 0.05^{b}
Commercial unhandled	-	14.5 ± 1.53^{cde}	$2.2\pm0.20^{\rm c}$	0.8 ± 0.07^{bc}

TABLE 4

Organ and condition indices calculated for fish at the conclusion of the 28 day growth trial. Superscript letters indicate significant differences between treatments within each performance indice (Tukeys HSD comparison). RGL data subjected to log transformation. (Values represent mean of 3 experimental aquaria \pm SEM).

Diet / Treatment	Basal / Ingredient	HSI^1	RGL ²	CF ³
PSFI reference restricted	100/0	1.7 ± 0.07^{gh}	0.6 ± 0.03^{abc}	1.7 ± 0.06^{bc}
PSFI reference satiated	100/0	$1.5\pm0.08 f^{gh}$	0.6 ± 0.01^{abc}	1.8 ± 0.05^{bc}
Extruded wheat	90/10	$1.7\pm0.04^{\rm h}$	0.5 ± 0.03^{ab}	1.8 ± 0.09^{bc}
Extruded wheat	80/20	1.4 ± 0.10^{efgh}	0.6 ± 0.03^{abc}	1.8 ± 0.04^{bc}
Extruded wheat	70/30	1.2 ± 0.01^{cdef}	0.6 ± 0.01^{abc}	1.8 ± 0.07^{bc}
Extruded wheat	60/40	1.3 ± 0.03^{def}	0.7 ± 0.01^{bc}	1.7 ± 0.08^{bc}
Pre-gelatinised starch	90/10	0.9 ± 0.10^{abcd}	0.6 ± 0.01^{abc}	1.7 ± 0.06^{bc}
Pre-gelatinised starch	80/20	1.2 ± 0.12^{cdef}	0.6 ± 0.01^{abc}	1.6 ± 0.11^{abc}
Pre-gelatinised starch	70/30	1.2 ± 0.03^{cdef}	0.6 ± 0.02^{abc}	1.7 ± 0.05^{bc}
Pre-gelatinised starch	60/40	$1.4\pm0.03^{\text{efg}}$	0.6 ± 0.02^{abc}	1.7 ± 0.05^{bc}
Diatomaceous earth	90/10	0.8 ± 0.01^{ab}	0.6 ± 0.06^{c}	1.7 ± 0.01^{bc}
Diatomaceous earth	80/20	1.3 ± 0.01^{ef}	0.6 ± 0.01^{abc}	1.6 ± 0.05^{abc}
Diatomaceous earth	70/30	1.1 ± 0.14^{bcde}	0.6 ± 0.02^{abc}	1.5 ± 0.05^{ab}
Diatomaceous earth	60/40	1.3 ± 0.03^{ef}	0.6 ± 0.03^{ab}	$1.3\pm0.10^{\text{a}}$
Commercial handled	-	0.6 ± 0.03^a	0.6 ± 0.00^{abc}	$1.9\pm0.06^{\rm c}$
Commercial unhandled	-	0.9 ± 0.02^{abc}	0.6 ± 0.01^{a}	1.8 ± 0.04^{bc}

¹ initial mean HSI of all diets \pm S.D= 1.12 \pm 0.05. ² initial mean RGL of all diets \pm S.D= 0.64 \pm 0.02. ³ initial mean CF of all diets \pm S.D= 1.45 \pm 0.06.



FIGURE 1

Relative weight gain of fish fed the restricted reference (open triangles), extruded wheat (closed circles), pre-gelatinised starch (closed squares) and diatomaceous earth (closed triangles) diets at 0,10,20,30 and 40% inclusion levels. (Data points and error bars represent the mean of three experimental aquaria \pm standard error).



FIGURE 2

Protein efficiency ratio of fish fed diets containing extruded wheat (closed circles), pre-gelatinised wheat starch (closed squares) and diatomaceous earth (closed triangles) at the 0, 10, 20, 30 and 40% inclusion contents. (Data points and error bars represent the mean of three experimental aquaria \pm standard error).



FIGURE 3

Relationship between crude protein intake and protein efficiency ratio (PER) of fish fed extruded wheat (open squares; solid line) ($R^2=0.0172$); pregelatinised wheat starch (closed squares; dashed line) ($R^2=2 \times 10^{-5}$) and diatomaceous earth (closed triangles; dotted line) ($R^2=0.8267$) across all inclusion levels. Each data point represents a mean of the 12 fish in each experimental aquarium.

5. **BENEFITS AND ADOPTION**

- The research strategy of Aquafin CRC was developed explicitly to deliver the essential technologies needed by the Australian finfish farming industry, as identified by CRC participants. The industry partners defined the major goals they believed a CRC could achieve, and clearly indicated the weight of effort which should be applied to each of these goals. These goals and weightings were first defined at a workshop of potential CRC participants in December 1999. Partners involved in the current project included Ridley Aquafeed Pty Ltd, Silver Beach Aquaculture and Anthony O'Donohue (trading as O'Donohue Sand and Gravel Pty Ltd). During the planning period for the project, the South Australian Marine Finfish Farmers Association and the Stehr Group of Companies (at that time involved in marine fish farming and tuna grow-out) contributed to research priorities and plans.
- The industry participants continued to refine their priorities during subsequent development. Anthony O'Donohue leased Silver Beach operations and assumed obligations to the CRC from Silver Beach. Industry participants and researchers met regularly (several times per year) to discuss results, implications of results and how they might be used, and refined plans for subsequent experiments.
- Production of temperate marine finfish (predominantly mulloway and yellowtail kingfish with minor contributions from other marine species) in Australia increased to 1,763 t worth \$14.3 million in 2005/06 (O'Sullivan et al., 2008). There was no reported production of mulloway or yellowtail kingfish prior to 2001/02 (O'Sullivan and Dobson, 2003). Production is primarily in South Australia. Estimates for production in 2006/07 and beyond, indicate a significant increase in both the quantity and value of production.
- The research reported here has contributed in several major ways to Australian marine fish aquaculture:
 - Information on ingredient digestibility and nutritional requirements for mulloway and yellowtail kingfish has helped nutritionists formulate diets for marine fish diets.
 - Bioenergetic models for mulloway and yellowtail kingfish will assist farmers to plan feeding strategies, accurately estimate feed requirements, predict nutrient outflows from their farming operations, model growth and production for marketing purposes and populate farm financial models. The bioenergetic models will assist feed manufacturers to formulate diets to meet requirements for digestible protein and digestible energy (for fish at different phases of the growth cycle).
 - One of the most significant benefits is the confidence among feed manufacturers to use alternative ingredients to fishmeal and fish oil in response to reductions in availability and/or increases in price. The progression towards least-cost formulation (as is industry practice for terrestrial monogastric animal feed formulation) relies on rigorous ingredient evaluation, accurate estimation of nutritional requirements and systematic validation of different formulations. The increase in the price of fishmeal has made substitution a higher priority for feed manufacturers and customers concerned with rapidly rising feed ingredient prices. Some farmers remain adamant that fishmeal based diets are essential to ensure market access for exports of Australian farmed marine fish and for improved performance during sub-optimal environmental (e.g. temperature) conditions. Data provided in this report allows the cost of that strategy to be calculated.
 - Hatchery practices and nursery technology were refined providing a much clearer understanding of the importance of abiotic factors (particularly photoperiod and light intensity), larval feeding strategies and diets on the cost-effective production

of mulloway and yellowtail kingfish fingerlings. Information on feeding behaviour and the development of sensory organs in mulloway provides hatchery managers with a physiological basis for new feeding strategies.

- Low-cost technology for extensive production of mulloway larvae in fertilised ponds has been developed.
- The development of successful research methods cannot be underestimated as a benefit of this project. Research methods for diet development, larval rearing and nursery production have all assisted in the design of new research to address similar problems for other species.
- Research capacity at an institutional (Industry and Investment NSW and Ridley Aquafeeds) and personal level has been expanded.
- The aquaculture industry will benefit from new PhD qualified researchers focusing on nutrition and larval rearing.
- Adoption of nutritional information has been made possible because of the involvement of Ridley Aquafeeds throughout the project. Mulloway and yellowtail kingfish farmed in South Australia are using Ridley diets. Information has also contributed to formulations for other species sold to Australian farmers.
- Hatchery techniques have been shared with other hatchery managers and technicians during Aquafin CRC workshops and conferences, Australasian Aquaculture conferences and during specific meetings of marine finfish hatchery managers and technicians. A new Seafood CRC/FRDC initiative to support a marine finfish hatchery network to run regular workshops, support technical exchanges, fund travel bursaries and develop training activities for technical staff will help ensure Aquafin CRC technology is adopted for the benefit of Australian industry.
- The regular research meetings between Industry and Investment NSW and industry participants contributed greatly to the timely exchange of information between researchers and industry and the increased understanding of major issues affecting each group. This partnership is a major benefit realised from the Aquafin CRC and will assist with maintaining a strong R&D base for temperate marine finfish farming.

6. FURTHER DEVELOPMENT

Despite the commercial investment in snapper farming at the commencement of the Aquafin CRC, snapper farming has not developed in Australia and, without exception, operators moved to faster growing species such as mulloway and yellowtail kingfish. This progression was recognised during the Aquafin CRC and nutritional research and research to improve production of fingerlings shifted from snapper to mulloway and yellowtail kingfish. The industry in South Australia has also reduced emphasis on mulloway with increased focus on yellowtail kingfish.

For both species, data produced during this project will be published in the scientific literature (much of it is already published or "in press") and it will also be packaged in a format suitable for feed manufacturers and farmers. The finalisation of bioenergetic models for both species will allow feed manufacturers to accurately formulate diets for different size fish. Although it is likely feed manufacturers will only manufacture a small number of different specification diets for any one species, because of the cost of changing formulations and the relatively small demand, as production increases, information provided from this project allows feed manufacturers to formulate additional diets. The availability of digestibility data for both species gives feed manufacturers confidence to change ingredients within diets while maintaining required digestible protein and digestible energy contents. This is particularly important given the very rapid increases in fishmeal prices and the global search for alternative ingredients. During the course of this project, the cost of fishmeal has increased by at least 100% and at times has been virtually unavailable in Australia because of reductions in production and massive increases in demand, particularly from China. This rapidly changing supply/demand relationship for fishmeal has major implications for marine fish farming, particularly in Australia, where low production reduces the ability to negotiate large volume purchases of fishmeal at globally attractive prices. This reinforces the priority for continued investment in fishmeal replacement research.

Looking further ahead, demand to replace fishmeal and fish oil will increase and the importance of ingredient substitution will become more pronounced. It is likely that additional research will be needed to fine tune our understanding of how different ingredients perform in high specification diets for marine species. The relatively small differences in performance of ingredients of different cultivars of the same species (for grain-based ingredients) and different types of rendered animal products will become more important. Continued research into digestibility and utilisation of these ingredients, conducted in conjunction with feed manufacturers, will be a priority.

In terms of nutritional requirements, the modelling conducted during this project allows feed manufacturers to confidently formulate diets for optimum performance during environmental conditions that also approach optimum. However, fish farmers have reported sub-optimal performance during adverse environmental conditions (e.g. low temperature) and have speculated that improved performance during these conditions might be possible using new, different diet formulations, possibly also using different ingredients. This is a future research priority. The imperative for future nutrition research is to ensure whole-farm economics is considered when modelling different nutritional and feeding strategies. A key aspect is to ensure research is conducted with large fish most relevant to farming operations.

This project has led to considerable improvements in hatchery technology for mulloway to the stage where fingerling production for this species is reliable and routine. Broodstock management (including controlled phototherm regimes) has allowed fingerling production at any time during the year. Systematic research has determined the optimum combinations of photoperiod and light intensity for different age (size) larvae and juveniles and established optimum feeding regimes for larvae and juvenile to reduce feed and feeding costs.

Looking ahead, the key problem with yellowtail kingfish hatchery production is the high level of malformations. New research within the Seafood CRC is underway and is urgently needed to reduce levels of malformations to below 10%. This research will need to concentrate on nutrition and feeding, additional abiotic and biotic factors as well as innovative methods for screening malformed fingerlings at early sizes.

As the number and size of temperate marine fish hatcheries in Australia increases, the need to effectively share technology also increases. There is a strong need for more effective communication among hatchery technicians and managers to share advances and continually reduce the real cost of fingerlings. A marine fish hatchery network with regular meetings has been established.

7. PLANNED OUTCOMES

1. Profitable, sustainable and increasing industry for temperature marine finfish aquaculture in Australia. Achieved.

When the research programs for the Aquafin CRC were being designed, the key temperate marine finfish species being farmed or considered for farming in Australia was snapper (New South Wales, South Australia, Western Australia and Queensland). Research into mulloway was also underway on a small scale in New South Wales and South Australia. In addition, research into larval rearing for stripey trumpeter as an alternative species for salmonid farmers in Tasmania was underway. During the course of the Aquafin CRC, farmers who were culturing snapper were disappointed with slow growth rates and keen to trial other species. Research success with breeding mulloway and later yellowtail kingfish, made production trials with these species possible and farmers have progressively moved from snapper to mulloway to yellowtail kingfish. Production is concentrated almost entirely in South Australia although there are small operations in New South Wales and new, large farms being developed in Western Australia. Production trends are illustrated by the Figures below (compiled from Austasia Aquaculture Trade Directories from 2001/02 to 2008). Recent advice is that production of mulloway in South Australia is likely to decline as farms increasingly shift their production to yellowtail kingfish and focus on production of southern bluefin tuna. Production estimates for yellowtail kingfish for 2006/07 exceed 2,000 t.



Production estimates (tonnes)



Production estimates by value (\$AUD)

2. Viable hatcheries, culturing high-quality, low-cost temperate marine finfish (for aquaculture or stock enhancement e.g. mulloway). Achieved.

Commercial hatcheries for marine fish are operating in South Australia, Western Australia, New South Wales and Queensland. The largest commercial hatcheries are operated in South Australia. In addition to commercial hatcheries, government owned hatcheries are operated in Western Australia, South Australia, New South Wales and Queensland. Contracts to produce fingerlings for commercial operations have been negotiated at all government hatcheries on a regular or irregular basis. Techniques for hatchery production of temperate marine species such as snapper, mulloway, yellowtail kingfish and stripey trumpeter have improved considerably over the period of the Aquafin CRC, particularly as a result of the projects on those species and communication among scientists and technicians working in the hatcheries. Communication has been facilitated by the Aquafin CRC meetings and workshops. The improvement is evident in higher survival rates, more consistent production and lower real costs on a per fingerling basis.

3. Reliable information on nutritional requirements for temperate marine finfish. Achieved.

For mulloway and yellowtail kingfish, bioenergetic modelling has been used to calculate requirements for digestible energy and protein (as well as allowing calculation of nutrient content in fish effluent and feeding rations for maximum growth). Estimation of requirements using bioenergetic modelling was confirmed using empirical dose-response studies for mulloway, increasing confidence in reported requirements. All experiments were designed in consultation with industry partner (Ridley Aquafeeds Pty Ltd) and results were made available as soon analyses were completed.

4. Reliable information on digestibility of available protein and energy sources for temperate marine finfish. Achieved.

For mulloway and yellowtail kingfish, apparent digestibility coefficients for protein, energy and organic matter have been determined for practical ingredients used in commercial and experimental diets. This information allows feed manufacturers to substitute ingredients

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(e.g. fishmeal) with other ingredients while maintaining a similar digestible protein and digestible energy content. This provides feed manufacturers with the data to respond to changes in ingredient availability and price and helps reduce feed price rises for farmers. The determination of digestibility data also allowed research to determine nutritional requirements.

8. CONCLUSIONS

- We do not recommend feeding mulloway on floating feeds.
- Commercial sinking diets not specifically formulated for mulloway containing approximately 46% crude protein (CP), 19.4% lipid, 26% nitrogen free extractives (NFE) and 8% ash with an overall gross energy density of about 23 MJkg⁻¹ diet were suitable in terms of optimising weight gain and feed efficiency.
- Commercial feeds containing approximately 48% CP, 20% lipid, 23% NFE and 9% ash with an overall gross energy density of 21 MJkg⁻¹ diet promoted rapid weight gain and acceptable food conversion ratio (FCR) in juvenile yellowtail kingfish reared at 22°C
- Organic matter, protein, fat and gross energy of two imported fish meal products were well digested by mulloway and kingfish (all > 80%). The protein digestibility of rendered animal meals such as meat meal, poultry meal and blood meal was also high, but digestibility of feather meal was low. The low overall digestibility of feather meal by both species may indicate this batch was subjected to overheating during the rendering process.
- Protein from extruded wheat was well digested by both species (>80%) and appears to be independent of inclusion level. The digestibility of pre-gelatinised wheat starch by mulloway was significantly better than extruded wheat, but there were stepwise reductions in organic matter and gross energy approximate digestibility coefficients (ADC's) of pre-gelatinised starch as inclusion levels were increased from 10 to 30% of the diet.
- The protein in soybean meal, dehulled lupins and whole field peas was well digested, but the energy digestibility of whole field peas was very poor and reflected the significant amount of carbohydrate (fibre) present in this product. Both mulloway and kingfish were better at digesting dehulled lupin meal than whole field peas, however solvent extracted soybean meal was more fully digested than lupin or peas.
- Dehulled lupin or solvent extracted soybean meal will serve as a useful protein or energy sources in diets for mulloway and kingfish.
- ADC's of carbohydrates for mulloway and kingfish are not additive.
- Injection of 1 g D-glucose kg⁻¹ BW into the peritoneal cavity of mulloway or yellowtail kingfish resulted in an immediate and prolonged elevation of plasma glucose levels indicating impaired glucose homeostasis when glucose is administered via the intra-peritoneal cavity.
- Based on standardised glucose tolerance tests, yellowtail kingfish appear to be more tolerant of carbohydrate (CHO) than mulloway.
- Yellowtail kingfish grew rapidly and recorded stable protein efficiency ratio (PER) when fed test diets containing high levels of extruded wheat and pregelatinised starch which suggests this species can efficiently utlise dietary CHO to spare dietary protein.
- Bioenergetic models were developed for mulloway and yellowtail kingfish. These holistic models can be used to predict growth, formulate practical diets based on digestible protein and energy requirements for different stages of growth, establish feeding tables and predict theoretical FCR. Models can be extended to estimation of waste/nutrient outputs from farms.
- The routine metabolic rate routine metabolic rate (RMR) of mulloway and kingfish increased with increasing water temperature. In addition, yellowtail kingfish have a RMR which is nearly double the RMR of mulloway.
- The temperature at which RMR of mulloway or kingfish was least thermally dependant on temperature was found to be 28.5°C and 22.8°C, respectively and were considered representative of the ideal or optimal temperature for rearing each animal.
- Mulloway stocked at a density of 20 fish cage⁻¹ were significantly smaller after 25 days than fish stocked at densities of 35 or 55 fish cage⁻¹, respectively.
- Mulloway fed twice each day ate significantly more than fish fed once a day, irrespective of the time of feeding. FCR was similar and significantly lower (better) in groups of mulloway stocked at 35 or 55 fish cage⁻¹ fish compared to fish stocked at 20 fish cage⁻¹.

• Juvenile mulloway (e.g. 17g) should be stocked at densities that exceed at least 4.08 kg m⁻³. Higher initial stocking densities may be possible, but the resultant impact on feed intake and FCR will need to be carefully considered (e.g. > 16.32 kg m⁻³).

9. APPENDICES

9.1 Intellectual Property

All information brought into this project or developed during the project is public domain.

9.2 Staff

- Dr Geoff Allan, Principal Investigator/Research Leader, Aquaculture and Port Stephens Fisheries Institute Director.
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- Mr Ben Doolan, Fisheries Technician, Port Stephens Fisheries Institute.
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 - Nicole Dooley
 - Gayle Rowney

9.3 The effectiveness of dietary supplementation with feed attractants on the feed intake and growth performance of juvenile mulloway (*Argyrosomus japonicus*) (Pisces: Sciaenidae). An honours thesis by Troy Harris (University of Newcastle)

ABRIDGED VERSION

THE EFFECTIVENESS OF DIETARY SUPPLEMENTATION WITH FEED ATTRACTANTS ON THE FEED INTAKE AND GROWTH PERFORMANCE OF JUVENILE MULLOWAY (ARGYROSOMUS JAPONICUS)

(PISCES: SCIAENIDAE)

Troy A. Harris

October 2006

A thesis submitted as partial fulfilment of the requirements for the degree of Bachelor of

Science (Honours) in the School of Environmental and Life Sciences,

University of Newcastle.

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ABSTRACT

Feeding trials were undertaken at Port Stephens Fisheries Institute (PSFI), Taylors Beach, NSW to determine the effectiveness of substances that could be used as supplements in a commercial fish meal based feed to improve feed intake and growth performance of juvenile mulloway (*Argyrosomus japonicus*). The two trials, each of nearly a months duration tested for differences among powder (green lip mussel powder (GLMP), AP20®, AP30®, betaine, krill meal, squid meal and a nucleotide) and liquid (Digest®, Yellow® and Gold®) feed attractants based on their performance in triplicate tanks with each tank containing 30 juvenile mulloway. Each attractant was added at 2% batch wet weight to Barramundi feed (Ridley AquaFeed Pty Ltd. Deception Bay Qld) which is typically used in aquaculture of mulloway.

Fish were fed to apparent satiation twice daily and water quality was maintained throughout with a mean temperature of 20°C and a salinity of 35ppt. Factors used to measure the effectiveness of each feed attractant included feed intake, weight gain, feed conversion rate (FCR), activity level ad feeding zone of mulloway. ANOVA demonstrated that the 5% significance level none of the above factors differed significantly with diet, thereby demonstrating that dietary supplementation with the potential attractants neither improved nor exacerbated the feed intake or growth performance of juvenile mulloway. It is suggested that the barramundi feed which is fish meal based and thus naturally contains feed attractants masked any potential effect caused by the added feed attractants. It is recommended that further studies are carried out to examine the effectiveness of the feed attractants in plant feedstuff based feeds for mulloway.

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ABBREVIATIONS

ABARE	Australian Bureau of Agricultural and Resource Economics
ADP	adenosine-5-diphosphate
AEST	Australian eastern standard time
ANOVA	Analysis of variance
BET	Betaine
C1	Control diet 1
C2	Control diet 2
CF	condition factor
DAFF	Department of Agriculture Forestry and Fisheries
Df	degrees of freedom
DGST	Digest®
DO	Dissolved oxygen
FAO	Food and Agricultural Organsitation
FCR	Feed conversion ratio
FMERF	Fisheries and Marine Environmental Research Facility
GLD	Gold®
GLMP	green lip mussel powder
GMP	guanosine-5-monophosphate
HSP-70	heat shock protein - 70
IMP	Inosine-5-monophosphate
KRL	krill meal
MABG	mean adjusted biomass gain
MHC	myosin heavy chain
mRNA	messenger ribonucleic acid
n	sample size
NSW	New South Wales
NSWDPI	New South Wales Department of Primary Industries
NUC	nucleotide
Pers. comm.	Personal communication
PIRSA	Primary Industries and Resources South Australia
PSFI	Port Stephens Fisheries Institute
Qld	Queensland
SA	South Australia
SARDI	South Australian Research Development Institute
SEM	standard error of mean
SQD	squid meal
UMP	uridine-5-monophosphate
YLW	Yellow®
w/w	wet weight
®	registered
	-

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1. INTRODUCTION

1.1 Aquaculture: worldwide and in Australia.

Aquaculture is currently the fastest growing animal production sector in the world, expanding at an average annual rate of about 11% since 1984, in comparison with 3% increase for livestock meat and 1.6% increase for capture fisheries (Francis et al. 2001; Wing-Keong, 2002). Aquaculture production is expected to continue to increase at a rapid pace to meet the seafood and other industry demands of a growing human population and to compensate for the shortfall in wild-caught fish due to the overexploitation of many wild fisheries (Figure 1.1). The Food and Agriculture Organisation of the United Nations (FAO) predicts that any further increases in global consumption of seafood is expected to be met by aquaculture (FAO, 2002; DAFF, 2006). The FAO also predicted that this trend would continue to the point that by 2030, global fish consumption will have increased from the current per capita rates of approximately 150 million metric tonnes (FAO, 2002; Watanabe, 2003; Austasia Aquaculture, 2006; DAFF, 2006). This will lead to aquaculture dominating fish supplies with less than half of the fish consumed coming from capture fisheries, as the annual sustainable yield of marine capture fisheries is no more than 100 million metric tonnes (Watanabe, 2003; DAFF, 2006).



FIGURE 1.1

World capture fisheries and aquaculture production for the period 1970 - 2000 (Source: FAO, 2002).

The Australian aquaculture industry has shown considerable growth in the period 1990 - 2001 (Figure 1.2). This growth is expected to continue into the future in order to take advantage of the increasing demand for seafood products (DAFF, 2006). In 2003/04, the industry was valued at \$732 million, accounting for almost one third of the total gross value production of the seafood industry (DAFF, 2006). Australia's worldwide reputation as a supplier of high quality and safe seafood produced in environmentally sustainable practices has allowed Australian aquaculture producers to target niche high- value domestic and overseas markets (DAFF, 2006). The increasing demands for Australian native species i.e. blue fin tuna (*Thunnus maccoyi*), barramundi (*Lates calcarifer*) and snapper (*Pagrus auratus*), and the close proximity to Asia and Pacific markets, together with the world-recognised excellent seafood quality and standards, puts Australia aquaculture producers in a position to capitalise on future world demand for high quality seafood (DAFF, 2006).



FIGURE 1.2

Australian aquaculture production for the period 1990 - 2001 (Source: ABARE, 2002).

1.2 Mulloway (Argyrosomus japonicus)

1.2.1 Distribution and habitat

Mulloway (*Argyrosomus japonicus*) belong to the Family Sciaendae and are naturally found in Australia, South Africa, Namibia, Madagascar, Mauritius, Korea, Japan and India (PIRSA, 2003). Wild-caught mulloway of Australia inhabit the central and southern mainland waters from the Burnett River and Bundaberg in Queensland to North West Cape in Western Australia (PIRSA, 2003). Although common in western Victoria, they are much less abundant between Melbourne and southern New South Wales and have seldom been reported from Bass Strait (PIRSA, 2003). Mulloway live in coastal environments, particularly estuaries, embayments, ocean beaches, rocky reefs and the continental shelf to a depth of 150m (Battaglene and Talbot, 1994). Adults are generally found close to the estuary floor or around shallow coastal reefs or rocky shores (Battaglene and Talbot, 1994). Mulloway are prevalent around the mouths of large rivers, eg. the Coorong at the Murray River mouth in South Australia and Broken Bay at the Hawkesbury River mouth in New South Wales, especially after periods of high summer rainfall (PIRSA, 2003). Although juveniles are known to be euryhaline, occurring in estuaries, embayments and nearshore coastal environments, however, little information exists on their spatial distribution within estuaries (Love and Langenkamp, 2003).

1.2.2 Growth and development

Mulloway reach maturity at a length of 75cm or 6 years of age, and live to approximately 30 years of age in which they attain a size of 2 metres and weigh 43kg (PIRSA, 2003). The growth rate of mulloway varies according to temperature and availability of natural food and is considered high until the age of 8 years (PIRSA, 2003). In the Clarence River (NSW), wild mulloway grow to 25cm by the age of 1 year, and to 60cm by the age of 2 years at which point they weigh 2kg (Love and Langenkamp, 2003). Mulloway feed throughout the water column with adults consuming sand crabs, prawns, worms, and a variety of fish including yellow-eye mullet (*Aldrichetta forsteri*), leatherjackets (*Monacanthidae spp.*), garfish (*Hemiramphidae spp.*), blue mackerel (*Scomber australasicus*), bony bream (*Nematalosa erebi*), tommy ruff (*Arripis georgianus*), pilchard (*Sardinops sagax*), yellowtail (*Trachurus novaezealandiae*) and juvenile mulloway (PIRSA, 2003).

1.2.3 Commercial and recreational fisheries

Mulloway are highly prized by both anglers and professional fishers (FMERF, 2003). The commercial and recreational catches in all Australian waters in 2000/01 were 500 t and 975 t, respectively (FMERF, 2003). The commercial catch in New South Wales has declined from 154 t in 1992/93 to 88 t (value: \$640,000) in 1997/98 (ABARE, 2002). Consequently, interest in the development of techniques for the production of mulloway to enhance wild stocks and for aquaculture of table-fish has increased. Decline in the number of mulloway in Australian waters is thought to be due to the pressure of commercial and recreational fishing as well as the reduction in flow from numerous rivers, particularly the Murray River, which affects the spawning potential of mulloway (FMERF, 2003; PIRSA, 2003).

1.2.4 Interest in the culture of mulloway

The rapid decline in the commercial and recreational catches of mulloway from Australian waters has led to a large amount of interest in growing mulloway in near-shore coastal or land-based facilities, either for human consumption or for restocking natural waterways for recreational anglers (Battaglene and Talbot, 1994). Mulloway are a good candidate species for commercial aquaculture because they possess many of the attributes considered essential for an aquaculture species, such as a wide natural distribution, euryhaline, high fecundity, fast growth and good food conversion rates. They are also well recognised in the domestic seafood market (Battaglene and Talbot, 1994; PIRSA, 2003).

1.2.5 Larval rearing and development

Recent trials in New South Wales and South Australia have found that mulloway grow well in a range of water temperatures and salinities in tanks, ponds and recirculating systems, providing an array of opportunities for brackish water farmers (Love and Langenkamp, 2003). Mulloway were first bred at NSW DPI Port Stephens Fisheries Institute (PSFI) in 1992, and since then, NSW DPI Fisheries have produced large numbers of fingerlings by using extensive and intensive rearing techniques (NSW DPI Fisheries, 2000). In the period 2001-02, approximately 120 000 fingerlings, valued at an average of 87 cents each, were produced in New South Wales by commercial hatcheries (NSW Fisheries 2003). Cultured mulloway larvae require a salinity range of 5 - 35ppt, and temperatures of $18 - 25^{\circ}$ C, although they will tolerate up to 30° C (PIRSA, 2003). The optimum salinity for the rearing of juvenile mulloway is approximately 5 - 12ppt (Fielder & Bardsley, 1999). Growth rates through the larval and juvenile stages are relatively rapid with the fish growing from 0.3mm to 1.7mm a day, depending on the system used (intensive or extensive, respectively) (PIRSA, 2003). With the successful development of hatchery techniques, larval fish can be ready for transfer to grow-out facilities within 30 - 40 days (Love and Langenkamp, 2003).

1.2.6 Grow-out of mulloway

Grow-out can occur in salinity from 5ppt to 35ppt in which mulloway can grow up to 1mm per day, making it possible to produce a 1kg fish in 14 - 15 months (PIRSA, 2003). Adult mulloway also show good growth rates at a range of temperatures from $15 - 30^{\circ}$ C, with grow-out being successful in sea cages, coastal earthen ponds, and recirculating aquaculture systems (PIRSA, 2003). Mulloway possess good attributes for grow-out in sea cages, i.e. mulloway grown in sea cages at Botany Bay, Sydney, reached a legal size of 45cm (i.1kg) within 26 months at ambient water temperatures (NSW DPI Fisheries 2000). This is mostly due to their inclination to form schools and their ability to adjust to confinement, which enables the inspection for diseases to be relatively easy (Battaglene and Talbot, 1994).

1.2.7 Diseases

Cultured mulloway are resilient to diseases, however problems are greatest at high salinities and high temperatures, i.e. 35ppt and 30°C (PIRSA, 2003). Epidemic diseases caused by organisms such as monogenean trematodes and ciliated protozoan, have killed juvenile and adult mulloway (PIRSA, 2003). Infestations of Amyloodinium sp. (an external parasite) are a serious risk to cultured

2003). Infestations of Amyloodinium sp. (an external parasite) are a serious risk to cultured mulloway (Tucker, 2000). The behaviour of heavily infested fish often changes rapidly and may include 'flashing' or rubbing their bodies on tank surfaces, loss of appetite, slow swimming and rapid or laboured gill movements (PIRSA, 2003). Prophylactic treatment is often used as a control measure for protozoans and trematodes (PIRSA, 2003).

1.2.8 The aquaculture potential of mulloway

Aquaculture of mulloway is considered to still be in its infancy. However, mulloway is very similar to the American red drum (*Sciaenops ocellatus*) and the white sea bass (*Gynoscion nobilis*) which have been successfully cultivated in large numbers in America for a number of years (Chamberlain et al. 1990; NSW DPI Fisheries, 2000). Red drum are very similar to mulloway in their life history and breeding requirements and strong similarities exist in the larval rearing of the two species (Chamberlain et al. 1990). Commercial grow-out of mulloway is primarily occurring in NSW and SA but the combined production in 2002/03 was only several hundred tonnes (NSW DPI, Fisheries 2005). On-land trials are occurring in the south east of South Australia and other places in Australia (Nel, 2001; PIRSA, 2003).

Market size of mulloway is 700g to greater than 1.0kg, however large mulloway (>2.5kg) are sold much more frequently and obtain the best price per kilogram (PIRSA, 2003). Smaller wild-caught mulloway have poorer consumer acceptance due to a soap-like texture and taste, although 1kg mulloway grown in sea cages and ponds have shown favourable results (NSW DPI Fisheries, 2000). In 1991–92, the average price for mulloway (also known as jewfish or butterfish) was \$5.85/kg at the Sydney Fish Market, and the average monthly prices at the Melbourne Wholesale Fish Market ranged from \$4.07 to \$6.90/kg (PIRSA, 2003). The current price of mulloway is between \$10 -\$15/kg. Mulloway from sea cages have been grown to market size and have been sold live to Sydney's Asian live-fish market for greater than \$14/kg (PIRSA, 2003). Mulloway have also been grown in ponds designed for prawns, with excellent market acceptance (Love and Langenkamp, 2003; PIRSA, 2003). Cultured mulloway are well received by consumers with chilled whole/gilled gutted fish attaining prices of approximately \$10/ kg (PIRSA, 2003). The majority of Australian consumers prefer white fleshed fish with few bones and a mild flavour, and the fact that cultured mulloway can satisfy these consumer preferences indicates that a substantial market exists for this product (PIRSA, 2003). Unfortunately, little is known of the export potential of mulloway (Love and Langenkamp, 2003; PIRSA, 2003).

Marine fish farming is increasing in NSW and has the potential for considerable expansion in the short to medium term (Love and Langenkamp, 2003). Whilst the limited numbers of suitable coastal sits for the farming of marine finfish may limit the growth of the industry initially, if inland saline groundwater proves suitable for accommodating marine fish, a new industry could develop quite rapidly NSW DPI Fisheries, 2000). Mulloway grown at the Waikerie Inland Saline Aquaculture Centre during trials carried out by the South Australian Research and Development Institute (SARDI) grew twice as fast as wild stocks because groundwater was at ac constant temperature (Austasia Aquaculture, 2006). These fish were recently sold as restaurant fare (Austasia Aquaculture, 2006).

1.2.9 Constraints to the growth of the industry

In terms of grow-out potential the development of a successful and profitable mulloway industry in Australia is currently constrained by several factors. These include a paucity of information on dietary requirements, feed suitability and appropriate feeding protocols (NSW DPI Fisheries, 2005).

At present, the only existing commercial feeds for mulloway are those designed specifically for barramundi or Atlantic salmon. Although these feeds produce acceptable results, it is unknown if they are nutritionally optimal, especially for rapidly growing fish (NSW DPI Fisheries, 2005). Even basic requirements, such as optimal protein to energy ratios of diets need to be elucidated, as does investigations of alternatives to fish meal and fish oil (NSW DPI Fisheries, 2005). In addition, mulloway farms located in Australian states such as South Australia are faced with extremely wide fluctuations in water temperature compared to those located in the more temperate climates of Australia. These temperatures can fall below 15°C in winter and exceed 35°C in summer (Mark Booth, *pers. comm.*). As mulloway are ectothermic (cold-blooded), their metabolism is greatly affected by temperatures above or below their thermal tolerance range. Extremes of temperature will often reduce feed intake and reduce growth, outcomes that are not beneficial to the producer (Mark Booth, *pers. comm.*).

This problem is not unique to mulloway farmers. Trout species grow at temperatures ranging from 5° to 20°C (Avault, 1996). Above and below these temperatures, growth all but ceases, causing some trout farms to stop all feeding at 4°C (Avault, 1996). Pompano (*Trachinotus carolinus*) tolerate water temperatures up to 33°C, grow best at 25°C, reduce their feeding at 17°C, stop feeding and go into shock at 12°C and die at 10°C (Avault, 1996). The channel catfish (*Ictalurus punctatus*) feed best at 28° to 32°C, hardly at all at 13°C, and cease when the water temperatures reach below 12°C, resulting in weight loss over winter (Avault, 1996). Juvenile red drum (*Sciaenops ocellatus*), which are closely related to mulloway, can live in water temperatures of 10° to 30°C, but perform optimally at 22° to 28°C (Chamberlain et al. 1990; Tucker, 2000). Feed intake and growth of red drum are decreased at <20°C and are negligible at <10°C (Tucker, 2000).

Water temperature is the single most important factor influencing fish growth, especially in fish from temperate regions (Avault, 1996; Sandifer et al. 1993; New, 1987; Tucker, 2000). It directly affects feed intake, feed conversion, reproduction, larval development, and the general well-being of culture species (Avault, 1996; Tucker, 2000). Fish, which are ectothermic (cold-blooded), consume less oxygen at lower temperatures. The decreased oxygen intake decreases metabolic rate and subsequent energy and protein requirements (Avault, 1996). It has been reported that a 10oC decrease in water temperature nearly halves the metabolic rate of fish (Avault, 1996; Tucker, 2000). Fish do not need to consume energy to maintain a steady body temperature and are more efficient in terms of energy use than terrestrial animals (New, 1987). A cultured fish species generally only consumes enough feed to satisfy its energy and protein requirements (Avault, 1996; Jobling, 1983; Jobling and Wandsvik, 1983; Fletcher, 1984). Consequently, extreme water temperatures place financial strain on the farmer in numerous ways. The time taken for the fish to reach market size is extended, potentially exposing the fish to additional stresses, which in turn leads to a higher susceptibility to disease, predation, etc. (Avault, 1996). Whilst the farmer tries to entice the mulloway to feed, the amount of wasted feed also increases, causing further costs and diminished water quality. A method commonly used to enhance feed intake is supplementation with feed attractants (Takeda, 1980a, b; Mackie and Mitchell, 1985; Ikeda et al. 1988; Takaoka et al. 1990; Takeda and Takii, 1992).

1.3 Artificially formulated feeds and the use of feeding effectors

The predicted future increases in aquaculture production will not be possible without an increase in the production of formulated feeds for the cultured fishes. Artificially formulated feeds play a crucial role in sustaining the continued expansion of aquaculture production, mainly because feed can make up 50% or more of the production cost of most aquaculture systems (Wing-Keong, 2002). Aquaculture feed production is currently one of the fastest expanding agricultural industries of the world, with annual growth rates in excess of 30% per year (Francis et al. 2001). The increasing cost of fish meal, the major ingredient in formulated feeds, has led feed manufacturers to partially or fully replace fish meal with protein rich terrestrial ingredients (Smith et al. 2005). This has resulted in the feeds being less attractive and/or palatable to the fish. Feed manufacturers have attempted to
improve the attractiveness/palatability of these feeds by adding commercial or natural feed additives that supposedly increase feed intake and assimilation by olfaction and gustation.

1.4 Fish chemoreception

Fish are immersed in their physical, biological and chemical environment, and their sensory systems are in continuous interaction with environmental perturbations. Consequently, non-volatile compounds with small molecular weights, such as free amino acids, nucleotides, and quaternary ammonium bases, are prominent compounds of fish olfaction and gustation and have been implicated in various behavioural roles (Hara, 1992a).

The chemical senses are the most ancient of sensory systems, having evolved 500 million years ago (Hara, 1992a). They are involved in mediating two very important functions for survival of the individual and the species: namely feeding and reproduction. Fish detect chemical stimuli through at least two channels of chemoreception – olfaction (smell) and gustation (taste). Although a third system (solitary chemosensory cells) that employs epidermal sensory cells, is currently being investigated, the evidence is scarce and incomplete. The current theory suggests that the skin and oropharyngeal surfaces are provided with a diffuse system of chemoreceptors related to, but distinct from the gustatory system (Hara, 1992a). A distinction between olfaction and gustation in fish is not always as clear as in terrestrial, air-breathing vertebrates as both olfaction and gustation is that gustation is involved in the detection, selection and ingestion of food and protection against noxious substances, and olfaction is involved in a generalised alerting response and is involved in specific pheromonal responses associated with fright, mating, spawning, territorial and homing behaviours (Marui and Caprio, 1992).

Olfaction takes place when chemical information is detected and transmitted directly to the central nervous system by bipolar neurons of cranial nerve I (Marui and Caprio, 1992). The olfactory organ in teleosts originates in an analge formed by the ectoderm, and the whole organ remains ectodermal throughout its formation (Hara, 1992a). Gustation takes place when chemical information detected by specialized epithelial cells (taste buds) is transmitted to the central nervous system by neurons of cranial nerve VII (facial), IX (glossopharyngeal) or X (vagus) (Marui and Caprio, 1992). The taste buds are endodermal in origin although the external taste buds are claimed to be of ectodermal origin (Kapoor et al. 1975). Taste buds can be found on the gills, barbels, fins, oral cavity and pharynx, as well as over the entire body surface of some species but not however on the tongue as found in higher vertebrates (Kapoor et al. 1975).

Sensory information about the chemical environment is transmitted to the brain by olfactory and gustatory receptor neurons through a series of molecular, membranous and neural processes in which odorant molecules bind to receptor proteins in the ciliary plasma membrane, enabling them to activate a G protein (Hara, 1992b). The activated G protein then activates adenylate cyclase (Hara, 1992b). The resulting increase in second messenger camp concentration opens ion channels in the membrane, or translocates ions directly, causing membrane depolarisation (Brand and Bruch, 1992). This ultimately leads to the generation of impulses that project to the brain where higher-level processing allows the discrimination of odours by the brain (Hara, 1992b; Satou, 1992).

1.5 Fish behaviour

When exposed to chemical stimuli associated with food, fish initiate food-search behaviour. Feeding behaviour is a stereotyped succession of behavioural components that can be differentiated into several phases: (1) arousal, (2) search, and (3) uptake and ingestion (consummatory) (Hara, 1992a). However, they are in reality a continuum without necessarily distinct transition (Jones, 1992). Fish rely upon information received through all sensory channels, with the relative importance of individual sense organs differing among species. Indeed, their relative importance is

determined by factors such as their feeding strategy, ecological niches, and other abiotic and biotic environmental factors (Pavlov and Kasumyan, 1990).

A wide diversity of fish species have been shown to use chemical signals in the search, location and ingestion of food (Hara, 1992a). There is, however, a question remaining as to how and which chemical components of feed attractants are used for food selection and ingestion (Hara, 1992a). This uncertainty is due partly to differences in the methodologies employed and partly to simultaneous participation of both olfactory and gustatory systems as the same stimulus often exerts different behavioural effects depending on whether it is received by olfactory or gustatory organs (Hara, 1992a). In previous behavioural studies, the distinction of senses involved has not always been clear due to the fact that some chemical stimulants may act as attractants via olfaction whereas others may act as promoters or enhancers of food intake or ingestion (Hara, 1992a; Borquez and Cerqueira, 1998). In the current study, a behaviour index adapted from Stradmeyer (1989) will be used to examine and classify the behaviour of mulloway, however a distinction of the senses involved in the detection of the potential feeding effectors will not be elucidated.

1.6 Feed attractants and their application to aquaculture

Numerous studies on feed attractants for aquatic animals have been undertaken in the last three decades (Hara, 1992b). However, there is little information on the relationship between olfaction, gustation and nutrition in fishes and possible application of feed attractants to aquaculture (Takeda and Takii, 1992; Papatryphon and Soares Jr, 2001). Although a variety of feed attractants have been identified from experiments conducted on a variety of fish species, most belong to one of the following groups of chemicals: (1) free amino acids, (2) nucleotides and nucleosides, (3) quaternary ammonium bases, (4) mixtures of the previous three with or without animal extracts (Takeda, 1980a, b; Mackie and Mitchell, 1985; Hara, 1992b).

1.6.1. Free amino acids

Although L-Alanine, glycine and L-proline appear to be the chief components of feed attractants for numerous fish species, mixtures of these free amino acids have been shown to be more effective than when single free amino acids are used (Takeda and Takii, 1992). There is great variability in the composition of the active amino acid mixtures for different fish species (Takeda, 1980a,b). L-Valine, L-tryptophan and a mixture of L-tyrosine, L-phenylalanine, L-lysine and L-histidine have been identified as feed attractants for red sea bream, *Pagrus major* (Ina and Matsui, 1980; Fuke et al. 1981), jack mackeral, *Trachurus japonica* (Ikeda et al. 1988) and rainbow trout, *Oncorhynchus mykiss* (Adron and Mackie, 1978), respectively.

1.6.2. Nucleotides and nucleosides

Inosine-5'-monophosphate (IMP), uridine-5'monophosphate (UMP), guanosine-5'monophosphate (GMP), adenosine-5'-diphosphate (ADP) and inosine, have been identified as feed attractants for certain fish species (Takeda and Takii, 1992). IMP showed noticeable feeding stimulant activity for yellowtail, *Seriola quinqueradiata* (Takeda, 1980a,b), turbot, *Scophthalmus maximus*, brill, *Scophthalmus rhombus* (Mackie and Mitchell, 1985), jack mackeral, *Trachurus japonica* (Ikeda et al. 1988) and marbled rockfish, *Sebasticus marmoratus* (Takaoka et al. 1990). In these fish, feeding activity was further enhanced by the addition of some amino acids to the feed. Japanese eel, *Anguilla japonica*, preferred a diet supplemented with a mixture of L-alanine, glycine, L-proline, L-histidine and UMP, to one supplemented with the amino acid alone (Takeda and Takii, 1992). UMP on its own also showed only minimal feeding stimulant activity for *A. japonica* (Takeda et al. 1984).

1.6.3. Quaternary ammonium bases

Glycine betaine (trimethylglycine) has been reported to act as a feed attractant for benthic feeders such as pinfish, *Lagodon rhomboides* (Carr et al. 1976), pigfish, *Orthopristis chrysopterus* (Carr et al. 1977), puffer, *Fugu pardalis* (Hidaka, 1982), Dover sole, *Solea solea* (Mackie et al. 1980) and red sea bream, *Pagrus major* (Goh and Tamura, 1980). The effects were also amplified by the addition of some amino acids to the feed. The sulphur analogue of glycine betaine, dimethylthetin, has also been reported to be an effective feed attractant for *S. solea* (Mackie and Mitchell, 1982).

1.6.4 Mixtures

Certain mixtures of amino acids, nucleotides and nucleosides, quaternary ammonium bases and/or animal extracts have been shown to increase feed intake and growth performance (i.e. weight gain, feed conversion rate, etc) in some species of fish. Kofuji et al. (2006) tested animal extracts that could be used as supplements in low-protein/high-lipid diets to enhance protein digestion in yellowtail, *Seriola quinqueradiata* during winter. It was shown that dietary supplementation with krill and squid extracts improved growth performance of yellowtail at colder water temperatures. This was due to the superior apparent protein digestibility and trypsin, chymotrypsin and pepsin secretions found in the group fed feeds supplemented with the feed attractants (Kofuji et al. 2006).

Xue and Cui (2000) tested the effect of betaine, glycine, L-lysine, L-methionine, L-phenylalanine and a commercial squid extract on feed intake of gibel carp, *Carassius auratus gibelio*, fed either a fish meal-based feed or a meat and bone meal-based feed. None of the feed attractants displayed enhanced feeding effects when used with the fish meal-based feed. However, all of the attractants showed feeding enhancing effects in the meat and bone meal-based feed group. From these results, the authors concluded that the basal feed in which the attractants are used in conjunction with, influences the effect of the attractant. On the contrary, Papatryophon and Soares Jr (2001) demonstrated that a mixture of L-alanine, L-serine, IMP, and betaine improved feed and growth performance of striped bass, *Morone saxatalis*, fed either a high-fish meal feed or a plant feedstuffbased feed.

1.7 Digestion, absorption and growth

Desirable chemical cues stimulate cephalic reflex responses and promote the digestive and metabolic functions for ingesting nutrients (Takii et al. 1986a). This has been found to occur in mammals (Giduck et al. 1987). Takii et al. (1986a) fed two groups of 30 Anguilla japonica (initial mean body weight of 78g) a diet with or without a feed attractant mixture, twice daily. The mixture consisted of L-alanine, glycine, L-proline, L-histidine and UMP. The basal feed was composed of white fish meal, -starch, pollack liver oil, and vitamin and mineral mixtures. Growth performance, feed efficiency, protein efficiency ratio, and energy and protein retention rates were found to be greater in the fish reared on the diet supplemented with the feed attractant mixture.

Postprandial changes in digesta weight, digestive enzyme activities, and the efficiency of dietary carbohydrate and protein digestion were also measured on the final day of the feeding trial in order to determine possible causes of the higher performance observed (Takii et al. 1986a). The total gastrointestinal digesta weight decreased immediately after feeding in the feed attractant diet group but did not decrease until 3 hours after feeding in the group fed only the basal feed. The gastric digesta weight decreased linearly with time in both dietary groups, whereas the intestinal digesta weight was found to be lower in the fish fed the diet flavoured with the feed attractant. Pepsin-like enzyme activity in the gastric digesta was twice as high 3 hours after feeding in the feed attractant diet group both 3 and 6 hours after feeding. The digestibility coefficients of carbohydrate and protein in the flavoured diet group were found to be greater by 3% and 10%, respectively (Takii et al. 1986a).

From these results, it was obvious that digestive activity soon after feeding was greater in *A. japonica* fed the feed attractant diet than in those fed the basal feed (Takii et al. 1986a). The enhanced secretion of gastric juices in *A. japonica* fed the feed attractant diet was believed to be the result of the cephalic reflex response to chemical stimulation by L-alanine, glycine, L-proline, L-histidine and UMP. Takii et al. (1986a) suggested that the higher levels of trypsin-like enzyme activities found in intestinal digesta of *Anguilla japonica* fed the basal feed were due to the possibility that pancreatic secretion of the enzyme may increase in compensation for the lower pepsin-like enzyme activity in gastric digesta. It was concluded that enhanced growth in *A. japonica* fed the feed attractant diet was due to increased functioning of absorption and digestion through the cephalic reflex by chemosensory stimulation.

Takii et al. (1986b) further examined the possibility of enhanced growth of *A. japonica* being indirectly attributed to more efficient nutrient metabolism. In order to test this hypothesis, the effects of dietary feed attractants on the metabolic enzyme activities relating to amino acids and carbohydrates were studied. The levels of six hepatic enzymes were found to increase in A. japonica after being fed the feed attractant diet as opposed to the basal feed. It was established that metabolism of carbohydrate and protein is enhanced at a relatively early stage of feeding in eels reared on the feed attractant diet. Takii et al. (1986b) suggest that the increased activities of hepatic enzymes might be related to the increased secretion of insulin during the early postprandial period. Furuishi (1983) found that plasma insulin levels in carp, *Cyprinus carpio*, red sea bream, *Pagrus major* and yellowtail, *Seriola quinqueradiata* do not increase until 3 hours after feeding. This indicates that nutrients absorbed soon after feeding may be excreted and not sufficiently utilised until about 3 hours after feeding, when serum insulin levels increase (Takii et al. 1986b). Therefore, the enhanced growth performances detected in eels reared on the feed attractant diet may be partly due to the increased secretion of insulin period (Takii et al. 1986b).

1.8 Study aims and rationale

Recently, greater emphasis has focused on the potential of feed attractants such as amino acids, nucleotides and nucleosides, quaternary ammonium bases and/or animal extracts to promote feed intake in fish with a view to enhancing or increasing growth and maintaining production efficiencies (Houlihan, Boujard & Jobling 2001). The research effort on feed attractants has become more relevant to the aquaculture industry as more fishmeal and fish oil, the two most common ingredients in the commercial diets of marine carnivores, are replaced with alternative feed ingredients such as rendered meals, oilseeds, cereal grains and legumes. The use of attractants has also become more important as fish producers culture new and well known species in more diverse locations, locations that often challenge each species with a unique set of physio-chemical conditions.

The feed intake and hence growth potential of mulloway grown in the cooler waters of south Australia during the winter months is reduced compared to mulloway grown in the warmer waters of NSW and other places at the same time of the year. This has disadvantaged south Australian farmers and extended the grow-out cycle of their mulloway substantially. The feed industry has proposed the use of feed attractants as a means of overcoming low feed-intake in mulloway during the winter period. However, no information on chemoreception and feed attractants is presently available for this species grown under culture conditions unique to Australian waters.

Therefore, the main aim of the present study is to determine whether the addition of 2% of a range of different feed attractants to a commercial barramundi feed can establish greater feed intake and subsequent growth of juvenile mulloway. The following hypotheses are proposed:

H₁: The external addition of 2% powder or liquid feed attractant (green-lip mussel powder, Digest®, AP20®, AP30®, betaine, krill meal, squid meal, a nucletide, Yellow® or Gold®) to a commercial sinking barramundi feed will affect feed intake and growth performance when fed to juvenile mulloway (*Argyrosomus japonicus*) reared at a constant temperature.

 H_0 : There will be no significant difference in feed intake and growth performance of juvenile mulloway (*Argyrosomus japonicus*) reared at a constant temperature and fed a commercial sinking barramundi feed coated with 2% powder or liquid feed attractant (green-lip mussel powder, Digest®, AP20®, AP30®, betaine, krill meal, squid meal, a nucletide, Yellow® or Gold®).

The overall goal of this research is to provide valuable information on the use of different feed attractants in commercial feeds for mulloway. Improved feed intake reduces feed wastage and pollution and improves feed conversion efficiency and growth performance. Both outcomes improve the productivity and efficiency of mulloway farming.

2. MATERIALS AND METHODS

2.1 Feed preparation

Twelve test diets were prepared at the NSW DPI Port Stephens Fisheries Institute (PSFI), Taylors Beach, NSW. Each diet plus attractant combination was prepared in approximately 5kg batches and all attractants were applied to pellets taken from a single batch of 4mm commercial barramundi feed by top-coating.

The first diet, hereafter known as "Control 1" was a commercial barramundi feed that received no further treatment (formula no: 87530V23, run no: 60031; 43% crude protein, 20% crude lipid, 16.9% carbohydrate, 10.1% ash, 8% moisture, 2.5% crude fibre, 1.6% total phosphorous, 1.1% available phosphorous) manufactured by Ridley Aqua-Feed Pty Ltd (Deception Bay, Qld).

The second diet, herafter known as "Control 2" consisted of the aforementioned barramundi feed top-coated with 0.58% w/w fish oil (Skrettings Pty Ltd, Rosney, Tasmania). Pellets were spray coated (Selecta Spray 5L multi-purpose sprayer, Canyon Pty Ltd) with warmed fish oil ($\approx 40^{\circ}$ C) while they were tumbled in a stainless steel cement mixer. The batch was mixed for a further 5 min following the application of the fish oil.

Each of three different liquid attractants was applied to the barramundi feed at a rate of 2% w/w. Liquid attractants were applied in the same manner as fish oil. Each of the five powdered attractants were top-coated onto the barramundi feed after the addition of 0.58% w/w fish oil as described for control diet 2. Fish oil was used to ensure adequate adherence of the powdered attractant to the barramundi feed. Each powdered attractant was applied to the pellets whilst the batch was tumbled in the cement mixer using a hand held sieve. Pellets were mixed for a further 5 min after the application of the attractant.

All diet batches were placed in sealed 8L buckets and frozen until required (-20°C). Between each batch process, the cement mixer and utensils used were cleaned with warm water and pyroneg (a neutral detergent commonly used in biological and surgical applications), and dried with absorbent paper towel.

All attractants were supplied by a commercial feed company (Ridley Aqua-Feed Pty Ltd, Deception Bay, Qld). Attractants were chosen because they were readily available, cost-effective or had been trialled on other fish species . They are as follows; green-lip mussel powder, Digest® (liquid), AP20® (a manufactured poultry-based feed attractant), AP30® (a manufactured vegetable-based feed attractant), betaine, krill meal, squid meal, a nucleotide, Yellow® (a liquid from Ridley Aqua–Feed Pty Ltd) and Gold® (a liquid from Ridley Aqua–Feed Pty Ltd).

The adherence of the attractant to the feed and the sinking rate of each dietary treatment was tested to ensure that the fish in each tank have equal opportunity to detect and capture the feed as it passed through the tank. Two pellets of each diet were dropped into a 60cm column of water and the time taken for each pellet to reach the bottom was recorded. Pellets were simultaneously observed to determine if any attractant was removed before either pellet reached the bottom. On review of these

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results (Table 2.1), the batch containing krill meal was re-made due to poor adherence to the feed. A mortar and pestle was used to regrind the krill meal into a finer powder before re-making this particular batch. The new batch was tested and found to have acceptable adherence.

TABLE 2.1.

The sinking rate of pellets (n=2) and the adherence (personal observations) of each attractant to the barramundi feed when dropped into a 60cm column of water (n=2).

Attractant	Sinking rate (secs)	Mean sinking rate (secs)	Adherence (pers. obs.)
Control1	7		
	7	7	
Control2	5		
	5	5	
GLMP	7		
	6	6.5	
Digest®	8		
	7	7.5	
AP20®	5		
	6	5.5	
AP30®	7		
	5	6	
Betaine	9		air bubble attached
	5	7	
Krill	6		flakes came off
	6	6	flakes came off
Krill (re-prepared	d) 7		
~	9	8	
Squid	9		air bubble attached
	6	7.5	
Nucleotide	13		air bubble attached
	6	9.5	
Yellow®	10	7.5	air bubble attached
0.110	5	1.5	
Gold®	1	7.5	
	8	1.5	

2.2 Experimental design

Two separate experiments were carried out. Each experiment was orthogonal, one factorial designed for interpretation using one-way analysis of variance (ANOVA) with diet type (i.e attractant) as a fixed factor.

2.2.1 Experiment 1 – The effect of powdered feed attractants on the feed intake and growth performance of juvenile mulloway

Eight diets were tested in experiment 1 using 24 x 170L cylindroconical tanks (Table 2.2). Each experiment tank was stocked with 30 juvenile mulloway and each of the dietary treatments was randomly assigned to 3 replicate tanks. The experiment was run for 28 days (i.e. 11 April, 2006 - 9 May, 2006).

TABLE 2.2.

Diet type		Tank Number	ſ
Control 1	5	17	20
Control 2	4	9	21
AP20	2	8	22
Betaine	15	18	24
GLMP	12	14	23
Krill	3	16	19
Nucleotide	6	11	13
Squid	1	7	10

The results of the random assignment of eight diets to the tanks used in experiment 1; n=3.

2.2.2 Experiment 2 - The effect of powdered and liquid feed attractants on the feed intake and growth performance of juvenile mulloway

On review of the results of a power analysis (Faul and Erdfelder, 1992) conducted on the feed intake data of experiment 1, the number of replicate tanks in experiment 2 was increased from 3 to 4. Six diets were tested in experiment 2 (Table 2.3) and each of 24 experiment tanks was stocked with 30 juvenile mulloway. In this experiment each of the dietary treatments was randomly assigned to one tank in each of 4 rows according to the lay-out of the laboratory. Experiment 2 was run for 26 days (24 May, 2006 - 19 June, 2006).

TABLE 2.3.

The results of the stratified random assignment of six diets to the tanks used in experiment two; n=4.

Diet type		Tank Number	
Control 1	6	10	16
Control 2	1	9	18
AP30	3	12	14
Digest	2	8	15
Gold	5	11	13
Yellow	4	7	17

The 10 attractants were divided based on their physical state (powders in experiment 1 vs. liquids in experiment 2), although a powder attractant diet (AP30) was also used in the second experiment to ensure ample replication of the six powders in the first experiment. A second control was used to control for the presence of oil used in the powder attractant diets, as fish oil could potentially act as a feed attractant.

2.3 Facilities

A 24 tank flow-through system was used at the NSW DPI Port Stephens Fisheries Institute (PSFI) for both experiments (Figure 2.1). Each experiment tank was totally isolated and influent water passed directly through each tank and was then discarded.



FIGURE 2.1.

A schematic representation of the twenty-four tank flow-through system located within a photoperiod controlled laboratory at the PSFI used in both experiments.

Estuarine water from Tilligerry Creek (a tributary of Port Stephens) was pumped through twin 20 m nominal cartridges (Onga C969) within a single housing (Pantera by Onga, Model C1500, Dega/Quiptron, Braeside, Victoria) into a 45,000L sealed reservoir. A sand filter was used to polish and recirculate water within the reservoir. Water was pumped from the reservoir into a combination header tank and bio-filter containing 25mm bio-balls. Water within the header tank was gravity-fed into a sump and then pumped through a sand filter and twin 20 m nominal cartridges (Onga C969) within a single housing (Pantera by Onga, Model C1500, Dega/Quiptron, Braeside, Victoria) before re-entering the header tank. Water from the header tank was gravity-fed to each of the 24 experiment tanks with individual tank flow-rates set at approximately 1L min-1 (Figure 2.2). Due to the extremely low pH level (5.5) of Tilligerry Creek on day 16 of experiment 2, the flow rate was reduced to 12L min-1, which was kept for the remainder of the experiment.

Water drained from the bottom of each tank via an out-flow pipe. A 10mm plastic grate sat at the bottom of the tank to prevent fish entering the out-flow pipe. A water filter housing (Aqua Pure AP-11, Cuno Pacific Pty Ltd, Sydney, New South Wales) was attached to the side of out-flow pipe. A 25mm rubber hose with a stop-valve was attached to the bottom of the housing. This setup allowed faeces and uneaten feed to settle out and be subsequently removed. The 24 tanks were each aerated with 2 x 25mm diffusing air-stones. Tanks were covered with 10mm black open-weave bird netting to prevent fish escaping. One Clipsal 3kw and two Clipsal 2kw heaters were introduced into the system (one in the header tank and two in the sump, respectively) at various times in order to maintain a near constant water temperature throughout each experiment (approx. 20°C). The tanks were subject to a 12h photoperiod via the use of eight 80W spotlights pointed toward the ceiling in order to diffuse the light.



FIGURE 2.2.

A schematic representation of the 170L experimental tank and attachments, showing the inflow and outflow paths, used in both experiments.

2.4 Technical procedures

2.4.1. Stocking procedure

Prior to stocking, juvenile mulloway (progeny of broodstock from the PSFI) were held in a recirculating aquaculture system containing a 10,000L tanks located in a greenhouse at PSFI. While in the system they were hand fed a commercial 4mm sinking barramundi feed (Ridley Aqua-Feed Pty. Ltd. Deception Bay, Old) to apparent satiation twice daily. At the commencement of stocking, the 10,000L tank was lowered to a depth of 36cm and dosed with ethyl-p-aminobenzoate at a concentration of 10mg L-1 (pre-mixed with ethanol to form a 10% solution) in order to mildly anaesthetise the fish. Once the fish exhibited symptoms of anaesthetisation, 100 fish were randomly selected and their weight (g) and standard length (mm) recorded in order to provide their condition factor. Afterwards, mulloway were hand selected in groups of 15, bulk weighed and transferred to one of the 24 x 170L tanks in the laboratory (mean individual fish weight = 58g). This process was repeated until all experiment tanks contained 30 mulloway (720 in total). In the second experiment, mulloway were individually weighed (mean weight = 78g) and placed in buckets of 15 and transferred to their respective 170L tank. This process was repeated until all tanks contained 30 mulloway (720 in total). Individual fish weights were taken at the commencement of the second experiment in order to minimise the possibility of establishing a feeding hierarchy within a single tank due to large differences in fish size, which may have occurred in the first experiment. Thirty fish were placed in a mortality-replacement cage located in the greenhouse as stocking density was maintained in each tank by replacing dead fish with caudal fin-clipped fish of similar weight. As a contingency against stocking mortality in each experiment, 30 fish from the same group were placed in a replacement cage located in the greenhouse. In addition, any fish that died during experiments were replaced with a caudal fin-clipped fish of similar weight to ensure that stocking density was maintained during the experiment.

2.4.2. Experimental feeding and husbandry

The mulloway were fed to apparent satiation twice daily at 900 and 1500 hours AEST. A single 500mL feed container and teaspoon was used for each tank. The spoon was used to scoop pellets out of the feed container and into the tank to avoid contamination from one tank to another. The teaspoon held approximately 3g of pellets. The weight of each feed container was taken after being filled and at the end of each day to determine the amount of feed offered to the fish. At the commencement of each feeding, the activity level of the school of 30 mulloway in each tank was observed whilst the first 3 teaspoons of feed were being offered. Any uneaten feed was collected via the 25mm plastic hose with stop-valve attached to the bottom of each tank (Figure 2.1) and dried out for 16 hours in an oven (Qualtex, OM24T, 5.5 Amps, 1250 Watt, Watson Victor Ltd.) set at 105oC (methods specified by AOAC, 1990) on a weekly basis. The uneaten feed was then weighed using analytical scales (OHAUS Analytical Plus, AP250D, 210g x 0.1mg / 50g x 0.01mg). The apparent health of the fish was observed at each feeding. Any moribund or dead fish were removed from the tank, weighed, disposed of humanely and replaced with caudal fin-clipped fish of similar weight.

2.4.3. Harvest procedure

At harvest, each of the 170L tanks was lowered to a depth of 30cm and dosed with ethyl-paminobenzoate at a concentration of 10mg L-1 (pre-mixed with ethanol to form a 10% solution) in order to mildly anaesthetise the fish. Once the fish exhibited symptoms of anaesthetisation, each fish was weighed. In addition, every second from each tank had its length recorded in order to determine its condition factor. The fish were then placed in a 10,000L tank of clean sea-water located in a greenhouse at the PSFI in order to bring the fish out of anaesthetisation.

2.5 Water quality

Monitoring of the water quality was undertaken daily on eight stratified-randomly chosen tanks (two tanks from each row). Water temperature, salinity, dissolved oxygen, and pH were measured using a water quality meter (Yeokal, Model 611 Intelligent or Horiba U-10, Japan, depending on availability). Ammonium levels were measured weekly using the colorimetric test (Aquamerck, Model 1.08024, Germany). An adequate supply of water was maintained via periodically filling the 35,000L reservoir. The four 20 m nominal filter cartridges (Onga C969) were replaced daily to maintain adequate removal of suspended solids. The tanks were cleaned on a weekly basis to remove any faeces and prevent the build-up of algae.

2.6 Measurement of feed intake and growth performance

A variety of techniques have been used to measure the role of chemosensory stimuli in the feeding behaviour of fish (Xue and Cui, 2001). Electrophysiological techniques have been widely used to study olfactory and gustatory reactions to a range of chemical stimuli (Hara, 1994; Kohbara et al. 2000). Although valuable information can be obtained, electrophysiological studies can only suggest whether the fish are able to sense a chemical compound using their chemoreceptor, which does not necessarily mean that the stimulus will result in changes in feeding behaviour or increased feed intake (Xue and Cui, 2001). Behavioural bioassays have been designed (Carr and Chaney, 1976; Mearns, 1985; Lokkeborg et al. 1995) and are particularly helpful when testing a large number of stimuli within short periods (Xue and Cui, 2001). However, the results are sensitive to unnatural disturbances and the behavioural response does not always result in increased feed intake (Xue and Cui, 2001). Methods based on quantification of feed intake are most advantageous, although only one treatment can be applied to each tank of fish (Xue and Cui, 2001). This means that a large number of replicates must be employed, which is not possible in this study due to technical and logistical constraints. Therefore, feed intake, growth performance indices and a behavioural bioassay will be used to assess the effectiveness of the potential feed attractants.

2.6.1. Feed intake

The facilities permitted the quantification of uneaten feed and therefore an accurate estimate of feed intake and feed conversion rate can be provided. At the beginning of each experiment, two 10g samples of each of the respective diets were dried for 16 hours in the oven set at 105°C and weighed in order to find their moisture content. The weight of the moisture was factored in to the uneaten feed weight data before any calculations were carried out.

The following formula was used to calculate feed intake:

Feed offered (wet weight) – uneaten feed collected (dry weight plus moisture content weight).

The feed intake data for each tank was divided by the number of fish in the respective tank in order to obtain the mean individual fish feed intake for each treatment.

2.6.2. Mean adjusted biomass gain (MABG)

The replacement fin-clipped fish were not included in estimates of biomass gain. The following formula was used to calculate mean adjusted biomass gain:

(final total biomass + weight of dead fish) – (initial total biomass + weight of replacement fish).

The MABG data for each tank was divided by the number of fish in the respective tank in order to obtain the mean individual weight gain for each treatment.

2.6.3. Feed conversion rate

The feed conversion rate (FCR) is a measurement of how efficiently a fish converts feed into tissue. The following formula was used to calculate the FCR:

(feed offered (wet weight) – uneaten feed collected (dry weight plus moisture content weight)) / ((final total biomass + weight of dead fish) – (initial total biomass + weight of replacement fish)).

2.6.4. Condition factor

The condition factor is a measurement of a fish's morphological condition (Table 2.5). The condition factor was measured after each experiment to ensure the mulloway were not subject to extraordinarily high levels of stress that can be associated with stocking, etc. The following formula was used to calculate condition factor:

weight / standard length³.

The cubed co-efficient was chosen after consulting Lima-Junior et al. (2002) as growth in weight of mulloway is proportional to growth in volume.

TABLE 2.5.

The condition factor index used in experiment one and two to determine the morphological condition of the juvenile mulloway.

Condition factor index	Comments
1.6	Excellent condition.
1.4	A good, well proportioned fish.
1.2	A fair fish.
1.0	A poor fish, long and thin.
0.8	Extremely poor fish, narrow and very thin.

2.6.5. Activity level

An activity score loosely based on an index adopted from Stradmeyer (1989) (Table 2.6) was given for each tank at each feeding. The observations were based on the behaviour of the majority (school) of mulloway whilst distributing the first three teaspoons of feed (approximately 3 grams).

TABLE 2.6.

The behavioural index used to assess activity level in both experiments; based on the index used by Stradmeyer (1989).

Score	Behaviour
4	Fish feeding at above average activity level; swimming at higher than normal speeds, consuming multiple pellets in quick succession, intensely competing with each other for the pellets with sharp-angled turns.
3	Fish feeding at normal activity level; constantly swimming at a moderate speed to capture pellets, moderately competing with each other for the pellets.
2	Fish feeding at below average activity level; swimming casually or not at all, only moving their heads to capture the pellets, not competing with each other for the pellets.
1	Fish capture the pellets however later reject them (spit them out).
0	No feeding behaviour shown.

2.6.6. Feeding zone

The vertical zone in which the school was feeding was also given a value (Figure 2.3). In the same way as for the activity level, observations were based on the behaviour of the majority (school) of mulloway whilst distributing the first three teaspoons of feed (approximately 3 grams).



FIGURE 2.3.

A schematic representation of the 170L tank and feeding zones.

2.7 Statistical analyses

All statistical analyses were performed using Statgraphics Plus V4.1 (Manugistics Inc., Rockville, USA). The data for individual feed intake, individual weight gain, FCR, activity level and feeding zone in each experiment were statistically analysed using a single factor analysis of variance (ANOVA, alpha = 0.05). Cochran's test was used to test for homogeneity of group variances, an assumption made when using ANOVAs. Post-hoc power analyses on individual feed intake (the primary performance indicator of interest) were performed using GPOWER (Faul and Erdfelder, 1992).

3. **RESULTS**

3.1 Water quality

The water quality parameters for each experiment are shown in Table 3.1.

TABLE 3.1.

The water quality data obtained in experiment one and experiment two. Data presented as mean and (range).

	Experiment 1	Experiment 2	
Temperature (°C) Salinity (ppt) pH	20.4 (18.6 - 23.3) 36.3 (35.1 - 36.9) 7.2 (7.0 - 7.5)	18.4 (16.7 - 20.9) 33.8 (32.3 - 35.3) 7.7 (7.0 - 8.1)	
DO (mg L-1) Total Ammonia (mg L ⁻¹)	5.9 (4.9 – 6.9) 0.2 (0.2 - 0.3)	$\begin{array}{c} 6.1 (4.8 - 7.6) \\ 0.3 (0.2 - 0.4) \end{array}$	

3.2 Experiment 1 – The effect of powdered feed attractants on the feed intake and growth performance of juvenile mulloway.

The individual feed intake, individual weight gain, FCR, activity level and feeding zone data for mulloway met the assumptions of ANOVA, with Cochran's test confirming homogeneity of variances within the treatments (Appendix A). The fish consumed an average of 1.0% of their body weight each day and maintained an excellent condition factor (Table 3.2). No mortalities occurred during this experiment.

The diet containing krill meal demonstrated a mean individual feed intake of juvenile mulloway of 18.9g over the course of the experiment and was above that of both control diets (18.6g and 18.2g over the course of the experiment) (Figure 3.1). However, the overall p - value of 0.53 indicates a non-significant difference between all diets (Appendix B). Figure 3.1 suggests that the large standard error of the mean recorded for some of the treatments (notably the nucleotide diet, AP20® diet, and Control1 diet) may have contributed to the non-significance.

Although the mean individual weight gain of mulloway fed the krill meal diet appeared to outperform all the other attractant-containing diets (Figure 3.2), growing a mean 25.5g in 28 days, the individual weight gain of mulloway was found to not differ significantly among diets (Table 3.3). The use of the GLMP resulted in the poorest individual weight gain (Figure 3.2).

No significant difference in mean FCR between the diets was found (p > 0.05) (Table 3.3). Betaine (0.71) appeared to possess the most efficient mean FCR followed closely by Control1 (0.72) and Control2 (0.73) (Figure 3.3). The FCRs recorded (0.71 - 0.76) are comparable to that recorded for similar-sized mulloway grown in cages at the PSFI (Mark Booth, *pers. comm.*). Again, the large standard error of the mean recorded for a few of the treatments is likely to have masked the occurrence of a slight significant difference between the diets (Figure 3.3 and Table 3.3).

TABLE 3.2.

Mean \pm SEM values for the different performance indices of mulloway fed different control or
attractant-supplemented diets in experiment one.

	Total feed	Total	Stock	Harvest	Daily feed	Harvest
	intake	MABG	weight	weight	rate	condition
	(g/tank)	(g/tank)	(g/fish)	(g/fish)	(%)	factor
Control 1	558.4	777.5	54.6	80.5	0.9	1.7
SEM	<i>16.2</i>	22.4	0.2	<i>0.8</i>	0.0	0.0
Control 2	546.8	748.2	54.6	79.5	1.0	1.7
SEM	<i>3.3</i>	29.0	0.1	1.0	0.0	0.0
GLMP	522.8	688.2	54.9	77.8	0.9	1.6
SEM	11.2	23.1	0.2	<i>0.9</i>	0.0	0.0
AP20®	542.1	723.4	54.6	78.7	1.0	1.7
SEM	17.8	<i>35.4</i>	0.1	1.1	0.0	0.0
Betaine	529.0	746.0	54.7	79.5	1.0	1.6
SEM	11.2	<i>10.2</i>	0.1	0.4	0.0	0.0
Krill meal <i>SEM</i>	565.7	764.8	54.8	80.3	0.9	1.6
	11.5	<i>9.4</i>	0.1	0.2	0.0	0.0
Squid meal <i>SEM</i>	542.5	719.7	54.5	78.5	1.0	1.6
	11.8	20.3	0.1	0.6	0.0	0.0
Nucleotide	538.0	731.7	54.6	79	1.0	1.7
SEM	27.0	<i>37.7</i>	0.1	1.1	0.0	0.0

No significant difference in mean FCR between the diets was found (p > 0.05) (Table 3.3). Betaine (0.71) appeared to possess the most efficient mean FCR followed closely by Control1 (0.72) and Control2 (0.73) (Figure 3.3). The FCRs recorded (0.71 - 0.76) are comparable to that recorded for similar-sized mulloway grown in cages at the PSFI (Mark Booth, pers. comm.). Again, the large standard error of the mean recorded for a few of the treatments is likely to have masked the occurrence of a slight significant difference between the diets (Figure 3.3 and Table 3.3).

Although the mean activity level of mulloway did not significantly differ when fed the different diets (p > 0.05) (Table 3.3), the mulloway fed the krill meal diet appeared to possess a higher mean activity level than that of other fish (Figure 3.4). The majority of fish within all tanks displayed normal feeding activity, constantly swimming at a moderate speed to capture the pellets. Intense competition and aggression, which mainly consisted of attempts to bite the other fish, was observed on some occasions for all treatments. This behaviour was more frequent in the mulloway fed the krill meal diet. Below average feeding was quite common and each of the treatments recorded a '0 - No feeding behaviour shown' score at least once during the trial. There were no observed instances where a fish captured a pellet and later rejected it.



FIGURE 3.1.

Mean \pm SEM individual feed intake (g) of juvenile mulloway fed different control or attractantsupplemented diets over the course of experiment one. Refer to text for control and attractant diet abbreviations.



FIGURE 3.2.

Mean \pm SEM individual weight gain (g) of juvenile mulloway fed different control or attractantsupplemented diets over the course of experiment one. Refer to text for control and attractant diet abbreviations.



FIGURE 3.3.

Mean \pm SEM feed conversion ratio of juvenile mulloway fed different control or attractantsupplemented diets over the course of experiment one. Refer to text for control and attractant diet abbreviations.



FIGURE 3.4.

Mean ±SEM activity level of juvenile mulloway fed different control or attractant-supplemented diets over the course of experiment one. Refer to text for control and attractant diet abbreviations.

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FIGURE 3.5.

Mean \pm SEM feeding zone of juvenile mulloway fed different control or attractant-supplemented diets over the course of experiment one. Refer to text for control and attractant diet abbreviations.

The mean feeding zone of mulloway fed the different diets was reasonably uniform and did not significantly differ between diets (p > 0.05) (Figure 3.5 and Table 3.3). The standard error of the mean was large for most diets (Figure 3.5), with each of the 24 tanks obtaining the full range of scores (1 to 3). The mean feeding zone of mulloway fed the Control2 diet was noticeably lower than the mulloway fed the other diets (Figure 3.5).

TABLE 3.3.

Summary table of *ANOVA* results for experiment one showing the effects of diet on the individual feed intake, individual weight gain, FCR, activity level and feeding zone of juvenile mulloway. No significant differences were detected (p < 0.05) (Appendix B).

		Individual feed intake	Individual weight gain	FCR	Activity level	Feeding zone
Source	Df	MS F-stat	MS F-stat	MS F-stat	MS F-Stat	MS F-Stat
Diet Residual	7 16	0.67 0.90 0.75	2.54 1.23 2.07	0.00 1.03 0.00	0.01 0.34 0.03	0.03 0.30 0.11

A post-hoc power analysis indicated that, given the range of group means with respect to individual feed intake was 1.5, and the estimate of pooled error variance for this test was 0.749, the current study had a 29% chance (power = 0.29) of achieving statistical significance using an orthogonal, one factorial ANOVA test (alpha = 0.05) (Figure 3.6). Increasing the sample size to seven replicates per treatment would have yielded an 80% chance (power = 0.80) of achieving statistical significance, given the observed effect size of 0.54 between the control and experimental diets (Figure 3.6). A minimum effect size of 0.95 (a difference of 0.95 standard deviations between the mean individual feed intake values of the control and experimental diets) was needed to achieve a power of 0.80 using three replicates per treatment.



FIGURE 3.6.

Power curve based on data for individual feed intake of juvenile mulloway in experiment one (effect size: 0.54).

3.3 Experiment 2- The effect of powdered and liquid feed attractants on the growth performance of juvenile mulloway

The individual feed intake, individual weight gain, FCR, activity level, and feeding zone data met the assumptions of ANOVA, with Cochran's test confirming homogeneity of variances within the treatments (Appendix A). The fish consumed an average of 0.5% of their body weight each day and maintained an excellent condition factor (Table 3.4). Survivorship 96.6%. Due to a technical error on day 21 which led to a dissolved oxygen deficiency in six tanks, only three replicates were used in the statistical analyses of the second experiment (the fourth replicate of each treatment was excluded).

Apart from the mean individual feed intake of juvenile mulloway fed the Digest® diet being relatively low (10.5g), the individual feed intake of the fish fed the remaining diets did not differ greatly (Figure 3.7). However, a significant difference between diets was not established (p > 0.05) (Table 3.5).

Despite the low variation in initial body weight (6.5%) and cumulative feed consumption (7.5%) over the experimental period, the fish showed a high variation in individual weight gain between all diets (Figure 3.8). The Yellow® diet and Gold® diet appeared to produce a greater weight gain than that of the remaining diets, though not significantly (p > 0.05) (Figure 3.8 and Table 3.5). The large standard error of the mean recorded for all diets likely prevented the finding of any significant differences between the diets (Figure 3.8).

TABLE 3.4.

	Total feed intake (g/tank)	Total MABG (g/tank)	Stock weight (g/fish)	Harvest weight (g/fish)	Daily feed rate (%)	Harvest condition factor
Control1	340.5	299.0	78.4	88.2	0.5	1.6
SEM	14.1	39.9	0.2	1.4	0.0	0.0
Control2	346.7	260.3	77.9	87.7	0.5	1.6
SEM.	22.0	44.6	0.2	1.3	0.0	0.0
Digest®	314.9	262.3	78.6	87.1	0.5	1.6
SEM.	9.1	36.7	0.4	1.2	0.0	0.0
AP30®	349.2	301.7	78.4	88.7	0.5	1.6
SEM	12.5	20.6	0.4	0.8	0.0	0.0
Yellow®	352.2	327.0	79.8	89.5	0.5	1.6
SEM	12.7	33.0	0.7	0.8	0.0	0.0
Gold®	362.3	325.3	78.3	88.8	0.6	1.6
SEM	9.4	26.0	0.2	0.9	0.0	0.0

Mean \pm SEM performance indices of juvenile mulloway fed different control or attractantsupplemented diets in experiment two.

The FCRs obtained in this experiment were slightly greater than one in all cases (Figure 3.9). Although not as efficient as the FCRs found in the first experiment, the least efficient feed conversion rate of 1.5 obtained by the mulloway fed the Control2 diet is still quite acceptable. The standard error of the mean appeared to differ between treatments, with mulloway fed either the Digest® diet, or one of the two control diets, resulting in large standard errors (Figure 3.9). Nevertheless, Cochran's test confirmed homogeneity of variances within the treatments (p > 0.05) (Appendix A). ANOVA again failed to detect any significant differences among treatments (Table 3.5).

The mean activity level of mulloway fed the Yellow® diet, Gold® diet, or AP30® diet appeared to be superior to that of both the control diets and the Digest® diet (Figure 3.10). However, ANOVA showed that the activity level did not significantly differ between the diets (p > 0.05) (Table 3.5). In contrast to the first experiment, the majority of fish displayed a below average activity level; swimming casually or not at all, only moving their heads to capture the pellets, and not competing for the pellets. Intense competition and aggression was not witnessed and each of the treatments frequently recorded a '0 - No feeding behavior shown' score. There were no observed rejections of pellets once the fish had captured the pellet.

The mean feeding zone of mulloway fed the different diets did not significantly differ between treatments (p > 0.05) (Table 3.5). The standard error of the mean recorded by the fish fed the Gold® diet was extremely large (Figure 3.11). The feeding zone used by the mulloway in this experiment was lower than that used by the fish in the first experiment. Feeding was not observed in zone 3 on any occasion throughout this experiment.



FIGURE 3.7.

Mean \pm SEM individual feed intake (g) of juvenile mulloway fed different control or attractantsupplemented diets over the course of experiment two. Refer to text for control and attractant diet abbreviations.



FIGURE 3.8.

Mean \pm SEM individual weight gain (g) of juvenile mulloway fed different control or attractantsupplemented diets over the course of experiment two. Refer to text for control and attractant diet abbreviations.



FIGURE 3.9.

Mean \pm SEM feed conversion rate of juvenile mulloway fed different control or attractantsupplemented diets over the course of experiment two. Refer to text for control and attractant diet abbreviations.



FIGURE 3.10.

Mean \pm SEM activity level of juvenile mulloway fed different control or attractant-supplemented diets over the course of experiment two. Refer to text for control and attractant diet abbreviations.



FIGURE 3.11.

Mean ±SEM feeding zone of juvenile mulloway fed different control or attractant-supplemented diets over the course of experiment two. Refer to text for control and attractant diet abbreviations.

TABLE 3.5.

Summary table of ANOVA results for experiment two showing the effects of diet on the individual feed intake, individual weight gain, FCR, activity level and feeding zone of juvenile mulloway. No significant differences were detected. (p < 0.05) (Appendix B).

		Individual feed intake	Individual weight gain	FCR	Activity level	Feeding zone
Source	Df	MS F-stat	MS F-stat	MS F-stat	MS F-Stat	MS F-Stat
Diet Residual	7 16	0.88 1.27 0.70	2.54 0.65 3.92	0.03 0.83 0.04	0.07 2.26 0.03	0.02 1.47 0.01

A post-hoc power analysis indicated that, given the range of group means with respect to individual feed intake was 1.6, and the estimate of pooled error variance was 0.697, the current study had a 30% chance (power = 0.30) of achieving statistical significance using an orthogonal, one factorial ANOVA test (alpha = 0.05) (Figure 3.12). Increasing the sample size to seven replicates per treatment would have yielded an 80% chance (power = 0.80) of achieving statistical significance, in light of the observed effect size of 0.59 between the control and experimental diets (Figure 3.12). A minimum effect size of 1.06 (a difference of 1.06 standard deviations between the mean individual feed intake values of the control and experimental diets) was needed to achieve a power of 0.80 using three replicates per treatment.



FIGURE 3.12.

Power curve based on data for individual feed intake of juvenile mulloway in experiment two (effect size: 0.59).

4. GENERAL DISCUSSION

The objective of this study was to identify which attractants from the range investigated had the potential to increase feed intake and subsequent growth performance of juvenile mulloway (*Argyrosomus japonicus*) at one constant temperature. Of the 10 feed attractants tested, krill meal, and to a certain degree, Gold® and Yellow®, acted as feed attractants in so much as they improved the growth performance of juvenile mulloway compared to other treatments. However, the increases observed in this study were not statistically significant. Therefore, the proposed alternative hypothesis; "H1: The external coating of a commercial barramundi feed with different types of powdered or liquid based feed attractants will affect the feed intake and growth performance of juvenile mulloway (*Argyrosomus japonicus*) reared at a constant temperature" is rejected at the 95% confidence interval.

Unlike the results of this study, numerous other studies have shown that supplementing fish feeds with feed attractants increases feed intake and enhances growth performance. However, the vast majority of feed attractant trials have used feeds containing low cost feedstuffs of low palatability (Takeda and Takii, 1992). For example, Borquez et al. (1998) presented amino acid-, betaine- and nucleotide-coated agar pellets to juvenile snook (Centropomus undecimalis); Papatryphon and Soares Jr (2000; 2001) used a mixture of amino acids, betaine and nucleotides when trialling a plant feedstuff-based feed for striped bass (*Morone saxatalis*); McGoogan and Gatlin (1997) used betaine supplementation with soybean meal-based feeds to enhance feed efficiency and growth of red drum (Sciaenops ocellatus); and Gomes et al. (1997) demonstrated that the addition of an amino acid mixture to soy protein concentrate-based feeds improved growth, feed intake and feed efficiency of European sea bass (*Morone saxatilis*).

The inconsistency between the results of the current study and the results of other feed attractant studies demonstrates the importance of testing attractants in commercially manufactured feeds, not merely identifying feed attractants for use in feeds that are potentially unpalatable to fish. Relatively few trials have been undertaken to assess the effectiveness of applying feed attractants to practically formulated feeds. Xue and Cui (2001) tested a mixture of amino acids, betaine and a commercial squid extract in both a meat and bone meal diet and a fishmeal diet of juvenile gibel carp (*Carassius*)

auratus gibelio). They found that a significant increase in feed intake and growth performance occurred when the attractants were added to the meat and bone meal feed but not the fishmeal-based feed. In fact, the inclusion of betaine and L-methionine to the fishmeal-based feed resulted in a significantly lower diet preference compared with that of the un-supplemented fishmeal-based feed. In the current study, the use of betaine, GLMP and Digest® also resulted in lower feed intake, albeit not significantly.

The non-significant effect of the krill meal, squid meal and nucleotide diets in the current study parallel the results reported by Kofuji et al. (2006). The feed intake and growth performance of yellowtail (*Seriola quinqueradiata*) fed a fish meal-based feed supplemented with krill extract, squid extract or a synthetic feed attractant mixture (2 amino acids and a nucleotide) did not significantly differ from that of the fishmeal based control diet.

The lack of significant differences among feed attractants in this study contrasts with the results of Castro et al. (1998) and Clarke et al. (1994). These studies found significant increases when using feed attractants in fish meal-based feeds of salmonids. However, in both cases the effect was attributed to an improvement in osmo-regulation (the energy required for osmo-regulation was reduced, therefore allowing fish to expend more energy into growth) rather than olfaction or gustation, as the increases in FCR, growth and survival only occurred only after the fish were transferred to seawater.

The general lack of an increase in feed intake and growth in fish fed a fish meal-based feed supplemented with feed attractants (as reported in this study) may possibly be attributed to the naturally high attractiveness of a fish meal-based feed (Avault, 1996; Li and Gatlin, 2006; Davis et al. 1995; Tucker, 2000; Xue and Cui, 2001). Fish meal contains known feed attractants such as free amino acids, urea, guanidine compounds, betaines, peptides, nucleotides, and quartenary ammonium compounds (Ruiter, 1995). As well as containing all the essential amino acids, fish meal is highly palatable and digestible containing high quality protein (protein digestibility: 2.7 -3.2), a reasonably high energy content and is rich in necessary vitamins and minerals such as B vitamins, phosphorous, calcium, selenium and long-chain n-3 polyunsaturated fatty acids (Ruiter, 1995).

It is evident that high quality fish meal can satisfy the nutrient requirements of most fishes, including mulloway (Avault, 1996; Ellis and Reigh, 1991; Davis et al. 1995; Gibson Gaylord and Gatlin III, 1995; Jirsa et al. 1997; Li and Gatlin III, 2006; Meilahn et al. 1996; Moon and Gatlin III, 1994; Reigh and Ellis, 1992; Tucker, 2000; Xue and Cui, 2001). In fact, studies have shown that the best performance of red drum (Sciaenops ocellatus), a species closely related to mulloway, is obtained when feeds containing fish meal are used (Ellis and Reigh, 1991; Gibson Gaylord and Gatlin, 1995; Jirsa et al. 1997; Moon and Gatlin III, 1994; Reigh and Ellis, 1992). Meilahn et al. (1996) reported that feed intake and growth in red drum decreased when the dietary fish meal content was reduced from 40% to 20% and 10%. Tucker et al. (1997) and Davis et al. (1995) obtained FCRs of 0.65 and 0.96 when red drum were fed a fish meal-based feed. These values are in accordance with the range of FCRs (0.71 - 1.33) obtained in this study.

When adequate levels of fish meal is not used in feeds, reduced growth can occur as a result of lower palatability and digestibility, amino acid and mineral deficiencies, indigestible oligosaccharides, and/or antinutritional factors (Tucker, 2000). Alternatives such as meat and bone meal, hydrolysed feather meal, and blood meal are less palatable and have an inferior balance of essential amino acids, and vegetable protein sources contain a relatively low level of protein, with some containing toxins (e.g. trypsin inhibitors) (Ruiter, 1995).

It has been shown that fishmeal based feeds are superior to terrestrial animal and plant-based feeds, even after the latter have been supplemented with feed attractants. Davis et al. (1995) stated that the use of a 40-60% fish meal-based feed resulted in greater feed intake and growth in red drum than feeds containing less fish meal and more soybean ingredients with seafood or shrimp flavouring.

Further evidence to suggest that a high quality fishmeal based feed does not benefit from the addition of feed attractants has been reported by Dias et al. (1997), Kubitza et al. (1997) and Singh et al. (2006). Dias et al. (1997) stated that superior feed intake and weight gain of European sea bass juveniles was observed in fish fed a plant protein-based feed with fish meal as the main protein source when compared to fish fed a corn gluten-based feed or one of two plant protein-based feeds with one of two soy protein concentrates as the main protein sources, with an amino acid mixture was added (2.5%) to the last three feeds. Singh et al. (2006) reported that five types of fish meal based feeds supplemented with Glycine, Proline and L-lysine. Kubitza et al. (1997) found that although dietary supplementation with a nucleotide (IMP: 2800 mg/kg) improved feed intake of largemouth bass (Micropterus salmoides) by 46% compared to the non-supplemented with either 2800 or 5600mg/kg IMP was lower than in fish fed a 10% fish meal-based feed.

Hence, it is evident that fishmeal is a very attractive ingredient when used in the diets of fish. In addition, supplementation of feeds containing adequate levels of fishmeal with feed attractants may do little to enhance feed intake because fishmeal itself may contain enough feeding effectors to "mask" the effect of any added attractants.

Although it is possible that the 2% inclusion level of the attractants in the current study may not have been adequate to initiate an enhanced feed intake or growth performance effect, several studies have obtained significant increases in feed intake and weight gain using lesser inclusion levels. Supplementation with 0.2% nucleotide enhanced feed intake and weight gain of striped bass (Morone saxatalis) (Papatryphon and Soares Jr, 2001), Toften et al. (2003) reported increased feed intake and growth of Atlantic salmon (Salmo salar) when 0.5% squid extract was used and Xue and Cui (2001) reported significant increases in feed intake and weight gain of Gibel carp (Carassius auratus gibelio) when fed a diet also supplemented with 0.5% squid extract. Likewise, betaine has been successfully used on numerous occasions at the 0.5% level (Papatryphon and Soares 2001; Xue and Cui, 2001; Felix and Sudharsan, 2004). Thus, the concentration of the different substances used in the current study cannot be considered insufficient.

Although a high quality fish meal based feed was used in this study, certain physiological factors associated with cold water temperatures can inhibit the fish's ability to intake large amounts of feed and prevent the fish from growing. This could therefore explain the lack of substantial feed intake and growth of fish in commercial aquaculture facilities during cooler periods. A study by Weber and Bosworth (2005) discovered that the mRNAs used to encode myostatin, myosin heavy chain (MHC), and heat shock protein-70 (HSP-70) were significantly affected by cold water temperatures, independent of feed intake. Each of these physiological indices have been shown to affect metabolism and catabolism, via regulating muscle growth and preventing muscle damage, respectively (Ferguson et al. 1986; Langley et al. 2002; Oishi et al. 2003; Overturf and Hardy, 2001; Thomas et al. 2000). Thus, unless the physiological and molecular mechanisms associated with fish growth are somehow manipulated, mulloway being kept below their optimal temperature range will likely continue to feed very little, regardless of the feed being offered.

Although additional water heaters were used in the second experiment, the mean water temperature in the second experiment was 2oC lower than the water temperature of the first experiment. This could help explain the differences observed in the performance of the two control diets in the different experiments. Thus, the higher mean water temperature of 20.4oC in the first experiment appeared to have resulted in the fish of the first experiment growing more rapidly than the fish of the second experiment. This is supported by a greater mean feed intake (18.4g vs 11.5g) and mean weight gain (25.4g vs 9.5g), as well as a more efficient mean feed conversion rate (0.72 vs 1.3) in experiment one when compared to experiment two. The fact that the fish also had a greater feeding activity level in the first experiment than in the second experiment (2.7 vs 2.1), suggests that the greater activity level is also related to the higher water temperature. The data also demonstrates that the mulloway in the first experiment fed at a higher zone than the mulloway in the second experiment, implying that the fish were more eager to feed and unwilling to wait until the feed reached a deeper zone in the tank.

The two experiments in this study possessed a 29% and 30% chance of achieving statistical significance in regards to feed intake, given the observed effect sizes of 0.54 and 0.59 standard deviation units (classified as medium effect sizes (Cohen, 1962)), respectively. However, from a practical perspective, a very sensitive test was not needed as a biologically significant effect size is of greater importance to this study and many other aquacultural studies (Sercy-Bernal, 1994; Thomas, 1997; Ling and Cotter, 2003). This is because the potential increases in production (i.e. weight gain) must counter-balance the added cost of incorporating the feed attractant/s into the diet. Effect sizes of 0.95 and 1.06 units were needed to achieve statistical significance at a power of 0.8, which is generally the minimum accepted level of power (Cohen, 1998; Thomas, 1997). In other words, this study would have an 80% chance of detecting a significant difference in feed intake between the diets, if the treatment means differed by approximately one standard deviation unit or more. Therefore, larger more biologically important effect sizes would have been detected if they had existed, resulting in a statistical significance.

Aquaculture growth trials are subject to large natural variations in the fish populations (Thomas, 1997). Even seemingly innocuous events such as people walking past the tanks can have an effect (Ling and Cotter, 2003). Researchers usually have only sufficient tanks to detect large differences (Ling and Cotter, 2003). However, the impact of this under-sampling although preferably avoided, is not of great concern when only large treatment effects are of scientific interest, as in the current study (Ling and Cotter, 2003). Unfortunately, the issue of practical significance is rarely addressed in comparative studies (Ling and Cotter, 2003).

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study demonstrated that the external addition of 2% of different feed attractants to a commercial barramundi feed containing fishmeal with different feed attractants did not improve nor exacerbate feed intake and growth of juvenile mulloway. It is highly probable that the commercial barramundi feed offered to the juvenile mulloway in this study was itself highly palatable and as such the addition of supplemental attractants or fish oil has had little effect.

The present study suggests that feeds containing fish meal, such as the commercial feed used in this study, are adequate to promote reasonable feed intake, growth and low feed conversion rates in juvenile mulloway grown in experimental tanks.

5.2 Recommendations for further study

In view of the importance of feed development in aquaculture, further research is encouraged on dietary supplementation with feed attractants to improve the palatability and nutritional value of non fishmeal based feeds. Fish meal has been the most desirable, albeit expensive, feedstuff for mulloway based on its palatability and ability to support rapid growth. However, as the aquaculture industry develops, the increased demand for fish meal is expected to raise the price of fishmeal, which has already increased from US\$600 t only one year ago to the current price of US\$1500 t (Austasia Aquaculture, 2006). As a result, it may soon become economically impractical to continue using fish meal in artificial feeds. Therefore, alternative feed ingredients, including the appropriate feed attractants and their inclusion levels, should be determined for the development of cheaper commercial feeds for mulloway. Particular attention should be applied to the use of krill meal and perhaps Gold® and Yellow® (both mixtures obtained from Ridley Aqua–Feed Pty Ltd) as these substances showed the most potential as feed attractants for mulloway.

It is also recommended that powerful, multifactorial experiments to examine interactions between potential feed attractants and feeds containing high or low fishmeal content be undertaken.

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APPENDICES

Appendix A: The results of the Cochran's tests

TABLE A1.

The results of Cochran's test performed on each of the variables for experiment one. * indicates significance. (p < 0.05).

Source		C statistic	p value
Individual	feed		
intake		0.404	0.21
Individual	weight		
gain		0.287	0.74
FCR		0.372	0.31
Activity leve	l	0.327	0.50
Feeding zor	e	0.284	0.77

TABLE A2.

The results of Cochran's test performed on each of the variables for experiment two. * indicates significance. (p < 0.05).

Source	C statistic		p value	
Individual	feed			
intake		0.452	0.30	
Individual	weight			
gain		0.273	1.00	
FCR		0.346	0.72	
Activity level		0.404	0.45	
Feeding zon	е	0.552	0.11	

Appendix B: The results of the ANOVA tests

Table B1. Analysis of variance on individual feed intake of experiment one.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups Within groups	4.71687 11.9789	7 16	0.673838 0.748679	0.90	0.5298
Total (Corr.)	16.6957	23		 -	

Table B2. Analysis of variance on individual weight gain of experiment one.

Source	Sum of	Squares	Df	Mean Square	F-Ratio	P-Value
Between groups Within groups		17.7754 33.1096	7 16	2.53934 2.06935	1.23	0.3444

23

50.885

Total (Corr.)

Table B3. Analysis of variance on feed conversion rate of experiment one.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups Within groups	0.00678333 0.0150667	7 16	0.000969048 0.000941667	1.03	0.4489
Total (Corr.)	0.02185	23			

Table B4. Analysis of variance on activity level of experiment one.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups Within groups	0.0825167 0.557067	7 16	0.0117881 0.0348167	0.34	0.9243
Total (Corr.)	0.639583	23			

Table B5. Analysis of variance on feeding zone of experiment one.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups Within groups	0.242117 1.81947	7 16	0.0345881 0.113717	0.30	0.9417
Total (Corr.)	2.06158	23			

Table B6. Analysis of variance on individual feed intake of experiment two.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups Within groups	4.41956 8.35913	 5 12	0.883912 0.696594	1.27	0.3386
Total (Corr.)	12.7787	17			

Table B7. Analysis of variance on individual weight gain of experiment two.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups Within groups	12.718 46.9962	5 12	2.5436 3.91635	0.65	0.6676
Total (Corr.)	59.7142	17			

Table B8. Analysis of variance on feed conversion rate of experiment two.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups Within groups	0.156361 0.453933	5 12	0.0312722 0.0378278	0.83	0.5544
Total (Corr.)	0.610294	17			

Table B9. Analysis of variance on activity level of experiment two.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups Within groups	0.341444 0.3624	5 12	0.0682889 0.0302	2.26	0.1146
Total (Corr.)	0.703844	 17			

Table B10. Analysis of variance on feeding zone of experiment two.

Source	Sum of Squares	Df	Mean Square	F-Ratio	P-Value
Between groups Within groups	0.07645 0.1252	5 12	0.01529 0.0104333	1.47	0.2714
Total (Corr.)	0.20165	 17			

9.4 Juvenile mulloway nutrition. An honours thesis submitted by Gayle Rowney (University of New South Wales)

Juvenile Mulloway Nutrition

ABRIDGED VERSION



A study of the digestibility of three animal meals and response to an acute glucose tolerance test.

Gayle Rowney

Supervisors: Dr Iain Suthers - UNSW Mark Booth – Port Stephens Fisheries Institute Stewart Fielder – Port Stephens Fisheries Institute

Submitted in partial fulfilment of the requirements for the degree of Bachelor of Science (Advanced Life Sciences), School of Biological, Earth and Environmental Sciences, Faculty of Science, The University of New South Wales

June 2004


The University of New South Wales Faculty of Science School of Biological, Earth and Environmental Sciences

Honours thesis project declaration page

I hereby declare that this submission is my own work and to the best of my knowledge it contains no materials previously published or written by another person, nor material which to a substantial extent has been accepted for the award of any other degree or diploma at UNSW or any other educational institution, except where the acknowledgement is made in the thesis. Any contribution made to the research by others, with whom I have worked at UNSW or elsewhere, is explicitly acknowledged in the thesis.

I also declare that the intellectual content of this thesis is the product of my own work, except to the extent that assistance from others in the project's design and conceptions or in style, presentation and linguistic expression is acknowledged.

Word count: 15864

(Signed).....Date....

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LITERATURE REVIEW Supplementing fish meal in aquaculture diets – problems and progress

ABSTRACT

Over the past decade, aquaculture has continued to advance faster than any other field of animal production in the world. In 2000/01, world aquaculture was estimated to have reached a value of US\$56.5 billion, and it is expected to continue to grow to meet consumer demand. As aquaculture expands the limits to many key fish diet ingredients is becoming apparent, and the cost-effectiveness of alternative ingredients must be assessed. Marine based ingredients, such as fish meal, are the most popular choice for protein sources, but are increasingly more expensive to produce. Fish meal already utilises 35% of the global fish catch, with around 4 kg of wet fish needed to produce 1 kg of dry fish meal. Consequently, if more than 17% fish meal is incorporated into a diet there is a net loss of fish protein from production.

In the efforts to find alternative sources of energy and protein for use in aquafeeds, international researchers have conducted studies to determine the potential of various ingredients in the diets of many established aquaculture species (e.g. barramundi, trout, silver perch, snapper, red drum). The first step in ascertaining the viability of an ingredient for use in a fish diet is the determination of its digestibility.

The aim of this study is to assess the digestibility of three protein sources of animal origin, fish meal, poultry meal and meat meal, in the diet of the Australian native marine finfish species, mulloway. Little is known about the nutritional requirements of this species, whose high fecundity, rapid growth and marketability make it potentially a very valuable species for aquaculture in Australia.

INTRODUCTION

Aquaculture is the world's fastest growing food production industry. Between 1984 and 1997, global aquaculture production increased 13% year-1 (Tacon and Dominy, 1999), and this rate is expected to continue to rise to meet the demands of a global population growing at a rate of 1.16% (Halver and Hardy, 2002; Tacon, 1998). In contrast, the seafood supply from wild capture fisheries has remained relatively static, with the average production rate increasing only 1.5% year-1 between 1984 and 1995 and 0.2% year-1 between 1996 and 1997 (Tacon and Dominy, 1999).

Though Australia has the third largest fishing zone in the world, its resources are less abundant and less productive than many other areas and the country is only ranked 55th biggest seafood producer by weight. For this reason, aquaculture is increasingly becoming an important source of seafood in Australia due to overexploited and less productive capture fishery resources. Though still in its infancy in Australia, aquaculture has been the fastest growing primary based industry over recent years (Shelley, 1999) with production increasing by 80% during the 1990's to a value of almost \$733 million in 2001/02, (ABARE, 2002). In comparison, Australian marine capture fisheries in 2001/02 were valued at around \$1700 million (ABARE, 2002). Though relatively small by world standards, aquacultures contribution to the total value of the Australian fisheries production has risen to around 25%, and is expected to increase in size four-fold to \$2.5 billion over the next decade (Brown et al., 1997; NSW Fisheries, 2002).

The increase in aquaculture production has brought about a higher demand for aquafeeds. In 1997, 11.5 million tonnes was needed to produce 12.9 million tonnes of feeding species worldwide (Tacon and Dominy, 1999). In the same year Australia produced 12.7 thousand tonnes of aquaculture product using formulated feeds. Compared to global production Australia produces very little aquafeed, with only 0.02 million tonnes aquafeed being produced locally in 1998.

In accordance with projected aquaculture industry growth, the global international requirements for aquafeed are expected to rise from 15 million tonnes in 2000 to 27 million tonnes by 2010 (Coutteau et al., 2000). However, fish meal production has been relatively static since the 1980s at 6-7 million tonnes and is not expected to increase as all current resources are fully exploited (Hardy and Kissil, 1997). As a result, a shortage in marine feed ingredients is expected within the next decade. This literature review aims to describe the current state of research into aquafeeds and feed ingredients.

Fish meal production

Aquafeed production has so far been dependent on fish meal and fish oil as cost efficient sources of dietary protein and energy, especially for carnivorous finfish species and prawns (Coutteau et al., 2000). In some formulated diets fish meal and fish oil may constitute up to 75% and 35% respectively of the total ingredients (Tacon and Dominy, 1999). Fish meals may be made from whole fish, as with anchovy, capelin or menhaden; or from processing residue, such as with whiting, pollock, herring and salmon. They have high levels of essential amino acids. Fat content levels range between 4 and 20%; ash content is highly variable ranging from around 11-12% in anchovy meal to more than 23% in whitefish meals made from filleting wastes (Table 2) (Hardy and Barrows, 2002).

Production of fish meal uses approximately 35% of the global fish catch (Tacon and Dominy, 1999), but the reliance on finite and valuable aquatic resources to feed cultured fish raises ecological and ethical questions and could jeopardize the long term sustainability of aquaculture systems (Coutteau et al., 2000; Kureshy et al., 2000). Additionally there is a growing economical concern about the uncertain market and escalating prices of fish meal.

Australia produces very little fish meal (Table 1) and the majority is imported from overseas. In 2001/02 Australia imported over \$32 million worth of fish meal. Because of our reliance on imports, Australia is particularly vulnerable to any world shortage of fish meal. The amount of fish meal produced by European nations is expected to be limited by guidelines recently introduced by the European Union regulating the quality of fish meals permitted for use in animal feeds. This is predicted to increase pressure on South American fish meal production, primarily from Peru and Chile. However, these supplies are unstable due in part to climatic variations in water temperature caused by El Nino. These fluctuations in fish meal production can cause prices to rise and result in a shortage of fish meal for aquafeeds (Alexis and Nengas, 2001).

Alternative feed sources

Feeding costs constitute as much as 70% of the total operating costs for aquaculture farms, so the search for viable alternative feed sources that are less expensive to produce has become an international priority (Wee, 1992). Dietary protein quality and quantity are of high importance in the culture of many fish species due to their high nutritional requirements. Dietary protein has profound effects on the growth

TABLE 1

Ingredient	Global Production (million tonnes)	Australian Production (million tonnes)	Australian production as a % of global production	
Fishmeal	6.8	0.01	0.15	
Terrestrial animal meals	5.5 ²	0.49	8.88	
Coarse grains				
Rice	609.9	1.10	0.18	
Maize	606.3	0.34	0.06	
Wheat	588.6	25.01	4.25	
Barley	128.2	5.04	3.93	
Sorghum	59.6	1.89	3.17	
Oats	24.1	1.01	4.19	
Oilseeds				
Soybeans	157.6	0.11	0.07	
Canola	43.2	2.43	5.63	
Sunflower seed	29.1	0.15	0.52	
Legumes				
Field peas	7.0	0.07	1.00	
Chickpeas	9.4	0.19	2.02	
Lupins	2.1	1.70	80.95	

Global and Australian production of selected products used in aquafeeds for the period 19991

¹ Data from FAO (2000)

² Global terrestrial animal meal data presented for the year 1997/98 (Allan et al., 2000a)

of an animal, feed utilization and the final composition of the whole fish. These factors all depend on the palatability of the feed, the indispensable amino acid profile, digestibility, and the presence of anti-nutritional factors or toxic compounds (Moon and Gaitlin, 1994). Feeds for carnivorous fish species usually contain 40 to 50% protein compared to 25 to 35% protein for omnivorous fish, such as catfish, tilapia, and carp, and for prawns (Hardy and Kissil, 1997).

Some Australian agricultural products are already used as aquafeed ingredients to supplement feed ingredients of marine origin. Compared to the production of fish meal, Australia has a high production of grains, legumes and terrestrial animal by-products (Table 1). Plant meals and terrestrial animal meals have already been identified as the most feasible alternatives to fish meal with considerable potential existing to increase their use both domestically and internationally (Hardy and Barrows, 2002).

Plant meals

Plant meals use a whole range of grains and grain by-products, ranging from high quality soybean meal to cereals like wheat and rice, for use in aquafeeds. The

TABLE 2

Typical composition of selected aquafeed ingredients3

Ingredient	Protein (g 100 g ⁻¹)	Lipid (g 100 g ⁻¹)	4 Total CHO (g 100g ⁻¹)	Gross Energy (MJ/kg)
Fish meals				, <u> </u>
Danish fish meal	72.9	11.4	-	21.5
Peruvian fish meal	70.2	11.3	-	20.9
<i>Terrestrial animal</i> <i>meals</i> Blood meal (spray dried)	94 9	_	_	23.9
Poultry meal	60.0	18.2	-	22.7
Meat and bone meal (lamb) Meat and bone	54.3	7.2	-	16.2
meal (beef)	49.2	9.2	-	16.1
<i>Plant meals</i> Soybean meal				
(solvent extracted) Lupins Lupinus angustifolius	47.8	3.7	40.5	17.0
(whole)	34.1	5.7	57.4	17.9
Field peas (whole)	25.5	1.1	70.0	17.0
Sorghum Wheat (Australian	14.5	-	83.2	18.8
Standard Wheat)	12.2	1.9	84.0	18.3
Wheat gluten	76.9	-	-	23.1

³ Data from Allan et al., (2000b)

⁴ Total CHO = Total carbohydrate (including fibre) (g 100 g⁻¹) calculated by difference = 100 - (protein + lipid + ash)

most important protein supplement produced from plants are the oilseed meals that are produced from the cake that remains after oil has been extracted from soybeans, cottonseed, canola, peanuts, sunflower and the like. These are all commonly available in Australia, with soybean meal probably the most widely used plant protein source in aquafeeds globally (Allan, 1997; Hardy and Barrows, 2002). Compared to fish meal, grains contain large amounts of carbohydrates (Table 2), including fibre and starch, some species have anti-nutritional factors, such as trypsin inhibitors, glossypol, glucosinolates, erucic acid, haemagglutinating agents, cyclopropenoic fatty acids and alkaloids; or are contaminated by mycotoxins produced by fungi (Allan, 1997). These can limit their digestibility and or the utilisation of the nutrients from them for some fish species (Allan et al., 2000b).

Fish species that are carnivorous by nature tend to digest plant materials poorly due to their reduced capacity to digest carbohydrate. Their gastrointestinal tract morphology is specialised for the breakdown of animal proteins, and as such are less able to digest carbohydrates, especially fibrous carbohydrates, in feedstuffs (Gaylord and Gaitlin, 1996; Hardy and Barrows, 2002). This has been attributed by many to the shortened gut-transit time that results in incomplete digestion and absorption (Wee, 1992; McGoogan and Reigh, 1996; Lee, 2002).

Many studies have shown that carnivorous species tend to use the dry matter and energy in animal products better than those from plant sources (Ebanasar, 1996; Gaylord and Gaitlin, 1996; McGoogan and Reigh, 1996; Lee, 2002). However, digestibility of plant meals with high carbohydrate contents can be improved through processing. The removal or reduction of carbohydrate, as in the wheat and corn gluten meals, can clearly result in improved dry matter and energy digestibility. The dehulling and refining of lupins can remove a significant portion of non-starch polysaccharide and improve dry matter and energy digestibility for this grain legume when fed to some species, for example silver perch (Allan et al., 2000b; Booth et al., 2001).

Some antinutritional factors can also be removed through processing. In the commonly used soybean meals, trypsin inhibitors can be inactivated by heat. Other antinutritional factors and toxins cannot be destroyed by heat, including glossypol in cottonseed meal, glucosinolates in canola meal, and phyletic acid in soybean meal, cottonseed meal, canola meal, other oilseed meals and some grain by-products. Suitable methods for testing for these compounds have been developed and can be used to assess toxin levels in feedstuffs (Hardy and Barrows, 2002).

Animal meals

Animal by-products are mainly derived from the meat-packing, poultry processing, and rendering industries. The protein content of these products is generally high, ranging from 50 to over 80% (Table 2) (Hardy and Barrows, 2002). The protein quality of animal by-product meals can vary depending on the quality and relative quantity of different waste products that are incorporated, the rendering equipment used and the way the meals are stored (Allan, 1997; Gaylord and Gaitlin, 1996). However, standards that minimise this variability have been established. These generally regulate protein quality through a set minimum pepsin digestibility level. The essential amino acid content of animal by-products meal is generally similar to that of whole egg protein, the standard by which protein quality is judged. They tend to be good sources of lysine, but poor sources of methionine and cystine, which are usually found to be limiting in diet formulations (Hardy and Barrows, 2002).

Meat meals are dried mammalian tissues, exclusive of hair, hooves, horn, hide trimmings, manure, and stomach contents, and generally have a protein content around 51%. Fat levels on average range between 9.1 and 9.7%; phosphorous levels tend to be below 4.4%. The calcium content is generally between 5.8 and 8%, but higher in meat and bone meals. Meat meal generally has a relatively high ash content of around 27% (Hardy and Barrows, 2002), though some companies have been able to decrease this to as low as 5% with a crude protein content of around 70% (Booth personal communication, 2003).

Poultry by-product meal is produced from waste from poultry processing plants, not including feathers or gizzard and intestine contents. After the chickens have been dressed, the remaining material is rendered and dried. In general, the protein content is approximately 58%, fat content about 13% and the ash content is cannot exceed 16%, of which the acid-insoluble content must be below 4%. Pet-food grade and low-ash poultry meals have a higher content and lower ash than regular poultry meals (Hardy and Barrows, 2002).

Feather meal can also be produced from the waste of poultry processing. Feather meal includes poultry feathers that have been hydrolysed under pressure in the presence of calcium hydroxide and

dried. It has a protein content around 80-85%, however its use in fish feeds is restricted due to its low protein digestibility by some fish species (Hardy and Barrows, 2002).

The protein content of animal meals tends to be higher than for meals of plant origin (over 50% and 20-50% respectively), and thus have a high potential for use in carnivorous fish diets. However, protein concentrates of plant origin, such as supplements derived from wheat and corn, can have higher protein contents (>60%), which can also be utilised in fish diets (Hardy and Barrows, 2002).

The main constraint on using terrestrial feed sources for fish is that compared to marine ingredients they are usually deficient in essential amino acids. Compared to fish meal with an amino acid index of 100, terrestrial plant derived protein sources are generally low in lysine (Lys; 20 to 85), methionine (Met; 20 to 80) and threonine (Thr; 55 to 85). Animal meals in general score higher but are often deficient in the same three essential amino acids (Tacon, 1998).

One other area of concern in using animal meals in fish diets is the transmission of transmissible spongiform encephalopathies (TSEs) such as mad cows disease (bovine spongiform encephalitis - BSE) and scrapie (ovine spongiform encephalitis). TSEs are caused by rogue prions called "protease resistant prions", which are proteins that lack a protease DNA. These transform other normal prions into their own image to spread within the infected animal, but researchers are still unsure of how it is transmitted. TSEs cause the "swiss cheesing" of brain tissues and is expressed in humans in the form of Creutz-Jacob Disease (CJD) and can be fatal. There is currently no evidence of TSEs existing in natural fish populations or if fish are possible carriers of the diseases (Meikle, 2002). Some studies are being carried out into the potential of fish contracting TSEs through feeding with infected meat, with studies to date showing no indications of replication of scrapie or BSE agents in experimental transmission studies (Meikle, 2002). The European Union has banned the use of terrestrial animal meals in aquafeeds and America has been considering the banning of imported seafood that has been fed on animal meals (Alexis and Nengas, 2001; Meikle, 2002).

Despite these concerns, animal meals still appear to have a high potential for use in aquafeeds especially in Australia. Not only are they more readily available and cheaper than fish meal in Australia, they have been shown to be well utilised by many

species of carnivorous fish (Ebanasar, 1996; Gaylord and Gaitlin, 1996; McGoogan and Reigh, 1996; Lee, 2002).

Mulloway

Mulloway (*Argyrosomus japonicus*; previously described as *A.hololepidotus*) are a large, estuarine sciaenid of recreational and commercial importance in Australia and South Africa (Fielder & Bardsley, 1999). It is widely distributed in the temperate waters of the African southeast coast, the entire southern seaboard of Australia, in the northern Indian Ocean it occurs off Pakistan and the northwest coast of India, and in the Northern Pacific from Hong Kong, along the Chinese coast, up to Japan and Korea (Griffiths and Heemstra, 1995).



FIGURE 1 Mulloway, *Argyrosomus japonicus*

Mulloway are a carnivorous species and limited studies have been done analysing gut contents of wild mulloway to determine their target prey. Juvenile mulloway in estuaries were found to feed mainly on teleosts, calanoid copepods, mysids, insects, amphipods and swimming prawns (Griffiths, 1997a; Fielder et al., 1999), whilst marine mulloway were found to feed on a wide variety of organisms, including benthic, epibenthic and pelagic crustaceans, cephalopods and teleosts, with teleosts being the principal item (Griffiths, 1997b). A marked shift in preference from prawns to fish has also been noted in estuarine mulloway as they increased in size (Fielder et al., 1999). Despite this, little else is known about the nutritional requirements of mulloway or their ability to digest and utilise different aquafeed ingredients.

Culture of mulloway

Interest in the culture of sciaenid fishes has grown rapidly over the past decade and a half. Several species including red drum (*Sciaenops ocellatus*), black drum (*Pogonias cromis*), spotted seatrout (*Cynoscion nebulosus*), orangemouth corvina (*Cynoscion xanthulus*) and white seabass (*Atractoscion nobilis*) have been successfully cultured for many years (Battalenge and Talbot, 1994). More recently, increased interest in mulloway has led to the development of successful breeding techniques. Mulloway larvae were reared for the first time by NSW Fisheries in 1993 using intensive rearing techniques. Weaning of larvae from live feeds to pelleted diets was successful and the mulloway were grown out to market size in sea cages (Fielder et al., 1999).

Mulloway have many traits that are favourable in an aquaculture species. It is highly fecund, euryhaline, grows quickly and the larvae are relatively easy to rear (Battalenge and Talbot, 1994). Trials assessing the use of both intensive and extensive rearing techniques have determined the best strategy to maximise survival and sustainable production of juvenile mulloway. Mulloway larvae and juveniles were found to grow over a range of salinities from 5-35 g/L. Trends suggested that in these early life stages, growth and survival were optimised at low salinities (5-12.5 g/L). These trials also found that the use of extensive larval rearing in large-scale ponds reliant on the propagation of natural zooplankton magnified by the use of fertilisers, was a successful method that required little labour input. Larval growth and survival was increased from 0.3-0.5 mm/day length increments in intensive tanks, to 1.2-1.7mm/day length increment in extensive tanks (Fielder et al., 1999).

As adults, mulloway have many advantageous traits for growout in sea cages. They are known to be gregarious and non-territorial compared to trout and salmon, readily form schools and adjust to captivity quickly, which makes inspection for relatively easy. However, the limited number of suitable coastal sites could restrict industry initially. Nevertheless, if research into the use of inland groundwater ponds for marine species proves successful, a new industry may develop relatively quickly (ABARE, 2003). Aquaculture production of mulloway in NSW in 2001/02 was reported in the NSW Fisheries Production Report to stand at 500 kg with 25 farms having permits to farm mulloway (NSW Fisheries, 2002).

Little growth data currently exists for mulloway. The species is known to reach a size of 25cm by the end of its first year and 60cm (with a weight of 2kg) by the end of its second year in the wild, but not much research has been done on the growth of mulloway in aquaculture systems. Mulloway have been grown out to market size, 45cm (1.1kg), in sea cages in 26 months at ambient water temperatures (ABARE, 2003). Additionally, preliminary studies have been conducted by NSW Fisheries on juvenile mulloway growing them in recirculation tanks for a period of 136 days. These fish were randomly assigned to two recirculation tanks for a period of 65 days, then graded and separated into small and large fish in different tanks till the end of the experiment (Figure 2). The specific growth rate, or daily instantaneous growth rate, of the mulloway used in this study was 0.91% day⁻¹ for all fish, with the faster and slower growing fish having specific growth rates of

0.99% day⁻¹ and 0.57% day⁻¹ respectively (figures 3 and 4) (NSW Fisheries – PSFI, unpublished data).



FIGURE 2

Mulloway weight vs. length (NSW Fisheries - PSFI, unpublished data)

Mulloway are also very important economically, being targeted by commercial and recreational fisherman and commanding high prices on the market (Griffiths and Heemstra, 1995; Fielder et al., 1999). In all southern mainland states mulloway are sold on the market as fresh, gutted fish. In 2003, mulloway has commanded an average wholesale price of \$11.50 per kilogram for wild fish and \$7.05 per kilogram for aquaculture product at Sydney Fish Market (Sydney Fish Market Website, 2003). A successful aquaculture industry for mulloway might help to decrease pressure on diminished wild stocks, provide sustainable employment, and reduce the amount of fish imported into Australia (Fielder et al., 1999).



FIGURE 3

Growth of mulloway, before size grading (NSW Fisheries - PSFI, unpublished data)

Breeding and culture techniques developed for mulloway are similar to those used for many other sciaenid species such as red drum and orangemouth corvina (Fielder & Bardsley, 1999). Studies show that there are strong similarities between the life history and breeding requirements of

mulloway and the commercially produced red drum (Fielder et al., 1999). Red drum has been commercially produced in America for many years and a great deal of research into their diet and nutritional requirements has been done to facilitate its aquaculture production. These studies provide some insight into what nutrient utilization and successful ingredient substitutions we can expect in the closely related mulloway.



FIGURE 4

Growth of mulloway, after size grading (NSW Fisheries - PSFI, unpublished data)

Red drum utilise fish meal very efficiently and grow more rapidly on fish meal than other protein sources (Table 3) (Moon and Gaitlin, 1994; Gaylord and Gaitlin, 1996; McGoogan and Reigh, 1996; Kureshy et al., 2000). It has also been shown that red drum digest the protein, lipid and energy of other animal feedstuffs very well.

TABLE 3

Percent apparent digestibility (ADC) of practical feedstuffs for red drum (Gaylord and Gaitlin, 1996).

Ingredient	Organic matter ADC	Crude Protein ADC	Lipid ADC	Gross Energy ADC
Select menhaden				
fish meal	93.9	87.9	87.2	95.0
Regular menhaden				
fish meal	93.7	76.9	67.6	92.1
Poultry by-product				
meal	75.6	48.7	59.0	71.7
Meat and bone meal	86.2	78.9	66.5	86.0
Soybean meal,				
dehulled	65.2	86.1	62.7	63.3
Cottonseed meal	70.2	84.5	75.4	70.4
Wheat	46.9	96.8	87.9	61.6

This ability to digest and absorb the nutrients in animal products more completely than those from plant products is accredited to their carnivorous nature. In the wild, red drum feed mainly on fish and crustaceans, which consist mainly of high levels of protein and lipid and low levels of carbohydrate. Their inability as carnivores to digest the carbohydrate in plant meals is most likely due to their physiological specialisation (well defined stomach with pyloric caeca and a relatively short intestine) (Gaylord and Gaitlin, 1996; McGoogan and Reigh, 1996).

The type of tissue from which protein is taken can be important in influencing growth, feed efficiency and protein efficiency ratio values in red drum. Moon and Gaitlin (1994) found that fish meal produced from fish skeletal muscle was better utilised than that produced from fish waste or whole body (feed efficiencies 0.97, 0.85 and 0.85 respectively). This is probably due to the low ash and high protein content in the skeletal muscle tissue compared to the other meals.

Feeding diets to red drum that are deficient in any of the indispensable amino acids generally results in a depressed appetite and a reduction in growth. Low palatability and poor amino acid profiles have been identified as the probable causes for the reduced feed efficiency and protein conversion efficiency observed in some species fed meat-and-bone meal, including red drum and rainbow trout (Moon and Gaitlin, 1994; Kureshy et al., 2000). It has been suggested that dietary protein supplements could be used to create amino acid balance in red drum diets (McGoogan and Reigh, 1996).

Measuring digestibility in fish

The first step in formulating artificial complete feeds for rearing animals under intensive conditions is an assessment of the availability of nutrients and energy in the Table 3: Percent apparent digestibility (ADC) of practical feedstuffs for red drum (Gaylord and Gaitlin, 1996) ingredients. Nutrient availability can be measured quickly and easily by determining the apparent nutrient or energy digestibility (Wee, 1992). Variation in the digestibility of the nutrients and energy in an ingredient is a major factor affecting their usefulness as energy sources to fish, since the main way ingested nutrients and energy are lost in fish species is through excretion as faeces.

Initially the measurement of feed and feedstuff digestibility requires collection of faecal samples. For aquatic species, this presents the challenge of how to separate the faecal material from water and avoid contamination from uneaten feed. These problems have led to the development of

methods for use with fish that are significantly different from those used for terrestrial animal and bird nutrition studies (Halver and Hardy, 2002).

The complete collection of faeces is very difficult in fish. Consequently, digestibility measurements using direct methods that require total collection of faecal material are rarely used for fish species. Measurements must therefore rely on representative uncontaminated faecal samples and the use of a digestion indicator to eliminate the need for quantifying the dietary intake and faecal output (indirect method). The inclusion of an inert marker, such as the commonly used chromic oxide, allows the calculation of digestibility coefficients of the nutrients based on the nutrient-to-indicator ratios in the diet and faeces (Windell et al., 1978; Halver and Hardy, 2002).

The sampling of faecal material from water has the added drawback of the potential leaching of nutrients and organic matter into the water column before collection. However, various techniques have been developed to try to overcome this problem, such as the use of faecal settlement tanks, stripping of the faeces and removal through anal aspiration or dissection. The digestibility values obtained have been proven to vary according to the collection technique used and as such, an appropriate method for the given conditions that facilitates the comparison of data with other studies must be chosen (Windell et al., 1978; Spyridakis et al., 1989).

Leaching of faecal soluble nitrogen compounds can occur in faecal collection tanks, which can lead to higher digestibility values. The extent depends on the efficiency of the collection method and nutrient solubility (Sugiura et al., 1998). Collection using a pipette found that the values were significantly higher than those obtained by stripping and dissection (Fernández et al., 1996). However, other studies have reasoned that the disturbance of the faeces during collection, such as by a pipette or net, can lead to excess of nutrients being leached into the water. For this reason specialised tanks that funnel faeces into a tube and out of the water flow have been used by some researchers (Cho et al., 1982; Sugiura et al., 1998; Allan et al., 1999).



FIGURE 5

Digestibility tank system based on faecal settlement collection techniques (NSW Fisheries - PSFI)

Passive faecal collection techniques are believed to be less stressful for the fish than the other invasive collection methods (Lee, 2002) and also to have the advantage of not having to kill fish to obtain results and being able to use many fish at once to obtain results (Austreng, 1978; Windell et al., 1978).

Collection of faeces through stripping encounters different problems such as collection of 'incompletely' digested materials and contamination of faeces, which can lead to inaccurately low digestibility values. Due to inability to control the total amount of faeces stripped it is easy to push out stomach or intestinal tract contents that have not been completely digested and absorbed. Additionally, faecal contamination can occur by forcing urine or sexual products out with the faeces or by increased digestive juices and mucus from handling stress. This leads to an increase in the amount of endogenous nitrogen material, including bile, enzymes, epithelial cells and mucus compared to that found when using a collection column and an overestimation of digestibility (Sugiura et al., 1998; Lee, 2002). Studies have also indicated that increased handling stress can lead to a reduction in feed intake and consequently a lower specific growth rate and higher mortality rate for these fish (Hajen et al., 1993).

The dissection method has the drawback of having to kill the fish. In addition to this, the digestibility values obtained through dissection tend to be highly variable. Studies have shown that the digestibility value tends to increase as intestinal contents are sampled closer to the anus (Fernández et al., 1996). This is due to digestion and absorption occurring along the entire intestinal tract far backwards in the rectum and as such, samples are recommended to be taken from as close to the anus as possible (Austreng, 1978).

Faecal settlement methods in seawater can result in a considerable amount of salt in the dried faeces, which can dilute the concentrations of all faecal constituents. Salt contamination will not affect the digestibility estimates for organic constituents, but it can affect the dry matter digestibility regardless of whether they are determined by an indicator or the complete collection technique. Some researchers have avoided this problem by using direct faecal collection methods such as stripping, anal suction and intestinal dissection; others have tried rinsing salt out of the sample, but this inevitably increases the risk of nutrient leaching losses (Hajen et al., 1993).

It is generally accepted that values for digestible energy and values for the digestibility of individual ingredients should be used to estimate levels of available energy and nutrients (as opposed to gross energy or crude nutrients) in feed ingredients for diet formulation. Sufficient information on digestibility values for common feed ingredients is now available to allow formulation of feeds on a digestible energy or digestible nutrient basis available for many species. An example of a typical formula and specifications for the long established salmonoid industry is given in table 4.

Determining the digestibility coefficients of practical feed ingredients for mulloway should provide insight into the nutrient utilization and enable more accurate ingredient substitution in diets designed for this species. Currently, there is no information to base diet formulation for mulloway on. The development of such a diet requires information on the animal's nutritional requirements, the appropriate presentation of nutrients in the feed and the animal's response to feed under different culture conditions (Wee, 1992). Currently, commercial feeds produced for established species, such as barramundi, are the most commonly used diets for feeding mulloway in aquaculture. Species specific diets can be formulated to meet different production goals, including rapid, efficient weight gain and successful maturation and reproduction (Hardy and Barrows, 2002).

TABLE 4

Ingredient	Percentage
Herring meal	28
Dried whey	5
Wheat germ meal	remainder
Corn distillers' solubles	4
Cottonseed meal	15
Pasteurized wet fish	30
Trace mineral premix	0.1
Vitamin and antioxident mix	1.5
Fish oil	6-6.75
Choline chloride (70% liquid)	0.5

Example of feed composition - typical formula and specifications for moist salmon pellets (Halver and Hardy, 2002)

CONCLUSION

The rapid growth of the aquaculture industry has made apparent the limits to many key fish diet ingredients and the need for alternative cost-effective ingredients to be assessed. International efforts to find alternative protein sources to fish meal has led to digestibility coefficients and growth data being produced for many established aquaculture species (e.g. barramundi, trout, silver perch, snapper, red drum). Due to variations in the protein utilisation and nutritional requirements of fish species, the formulation of cost-effective diets must be done on a species-specific basis. Currently, no data exists on the nutrient utilization and growth of mulloway.

THESIS Digestibility of animal meals and response to an acute glucose tolerance test

ABSTRACT

As the aquaculture industry expands it must contend with the rapidly approaching limitations to key feed ingredients and the environmental affects of aquaculture on the aquatic environment. Many of these issues can be addressed through nutritional research. Finding alternative feed ingredients to fish meal and fish oil has become a major priority for aquaculture nutrition research. Terrestrial feed sources such as animal meals and plant meals have a high potential for use in aquafeeds and in the formulation of cost effective diets for optimal production. This thesis looks at the nutrition of juvenile mulloway, a native Australian finfish that has the potential to be a highly profitable aquaculture species. Two main experiments were conducted into this species nutrition: a digestibility experiment examining three animal meals and an acute glucose tolerance test to give indications concerning the utilisation of carbohydrate. Mulloway were found to digest the organic matter, protein and energy of the three animal meals, fish meal, meat meal and poultry meal, fairly well (all >70%). The glucose tolerance testing showed that mulloway were not very efficient at absorbing and clearing D-glucose.

INTRODUCTION

In recent decades aquaculture production has been increasing at a faster rate than any other food production industry. Since 1992-93, the real value of Australian aquaculture production has more than doubled from \$331 million (in 2002-03 dollars) to \$743 million in 2002-2003. This corresponds to an annual rate of growth of 11% in nominal terms and 8% in real terms (ABARE, 2003). In general, high-value fish species are selected for aquaculture production due to their consumer acceptance and the attractive market prices. However high value species are, almost invariably, carnivorous by nature and generally require diets high in protein (Hardy and Barrows, 2002; Sabaut, 2002; Allan et al., 2003). With feed cost constituting up to 70 percent of the total running costs for an aquaculture farm the need to formulate cost-effective diets has become an international priority (Wee, 1992; Anderson, 2002). One main focus of this has been to lower the reliance on wild fishery produced fish meal and fish oil, which are both expensive and unsustainable (Coutteau et al., 2000). Alternative terrestrial protein sources such as animal meals and plant meals have been investigated as fish meal replacers.

Animal by-products are mainly derived from the meat-packing, poultry processing and rendering industries, such as meat meal, poultry by-product meal and feather meal. The protein content of animal meals tends to be higher than that of plant meals (over 50% and 20-50% respectively) and therefore have a high potential for use in carnivorous fish diets. In Australia, animal meals are more readily available and cheaper than fish meal, and have been shown to be well utilised by many species of carnivorous fish (Ebanasar, 1996; Gaylord and Gaitlin, 1996; McGoogan and Reigh, 1996; Lee, 2002). In 1999, Australia produced 8.88% of the global production of animal meals, compared to 0.15% of the global production of fish meal (FAO, 2000).

Plant meals use a whole range of grains and grain by-products including oilseed meals, soybean meals and cereals. The main limitations to use of plant meals in aquafeeds are their high carbohydrate contents and, in some plants, anti-nutritional factors. These can lead to lower digestibility and or utilisation of nutrients from them for some fish species (Allan et al., 2000b). Fish species that are carnivorous by nature tend to digest plant materials poorly due to a reduced capacity to breakdown carbohydrates, especially fibrous carbohydrates, in feedstuffs (Gaylord and Gaitlin, 1996; Hardy and Barrows, 2002). However, processing of plant meals can remove or reduce carbohydrate contents and improve digestibility for some species, for example silver perch (Allan et al., 2000b; Booth et al., 2001). Processing can also be used to remove antinutritional factors in plant

feedstuffs, such as inactivating trypsin inhibitors with heat, but is not effective for all antinutritional factors and toxins (Hardy and Barrows, 2002).

Many aquaculture nutrition research techniques have been developed to assess these alternative feed sources and to formulate diets that are both economical and efficient (Hardy and Barrows, 2002). Digestibility studies, growth trials and glucose tolerance testing are just a few of these methods that have been used effectively for various aquaculture species.

Digestibility experiments are used to assess the availability of nutrients from feed ingredients for individual species. Generally, in indirect determinations, a nutritionally balanced reference diet is combined with a test ingredient and an inert indicator (for example chromic oxide or ytterbium) to measure how much of the diet is digested (Windell et al., 1978, Halver and Hardy, 2002). Various methods have been developed to collect faeces for example settlement, stripping, dissection and anal suction. To minimise leaching of nutrients from the faecal pellets (which would lead to over estimated digestibility) collection methods that are fast and which minimise disturbance of faecal pellets are favoured (Cho et al., 1982; Fernández et al., 1996; Sugiura et al., 1998; Allan et al., 1999). Usually fish meal is found to be the most digestible ingredient in digestibility studies, but other animal meals, such as meat meal and poultry meal, and some plant meals, including wheat, lupins and field peas, have also proven successful in fish (Hardy and Barrows, 2002; Stone et al., 2003b).

The calculation of digestibility coefficients for both diets and individual ingredients gives a measure of the availability of nutrients within the feedstuffs (Wee, 1992). Variation in the digestibility of nutrients and energy in an ingredient is a major factor affecting their usefulness as energy sources to fish, since the main way ingested nutrients are lost in fish is through defecation.

Growth trials can be used to assess fish species growth rates on specific diets. Specific growth rates (also called the daily instantaneous growth rate) and feed conversion efficiency or ratios can be easily calculated from the weight gain of the fish, the length of time the experiment was run and the amount of diet fed over that time. This information can give an aquaculture farmer essential facts of food biomass and growth (Hardy and Barrows, 2002).

Increasing effort is being made to use more plant ingredients in aquafeeds. The main limitation to this is the ability of some fish to digest carbohydrates present in plants. Plant carbohydrates may be classified as either reserve polysaccharides or structural polysaccharides. The polysaccharide starch is a major energy reserve in most grains and legumes, and one of the principal components of wheat (~80%) and field peas (~40%). When completely digested glucose is one of the major breakdown products of starch (Allan et al., 2003) due to the structure of starch being made up of α -glucose monomers. The major structural polysaccharide in plants is cellulose, which makes up the tough plant walls and is the most abundant organic compound on earth. Though cellulose is also made of a monomer of glucose, β -glucose, the three-dimensional shape and therefore the properties of cellulose are very different from starch. The enzymes that digest starch by hydrolysing the α bonds are unable to hydrolyse the β linkages making it essentially indigestible by vertebrates (Campbell, 1996).

A quick and relatively cheap way to screen a species' ability to use dietary carbohydrate is by a glucose tolerance test. Carbohydrate, such as glucose, is introduced orally or injected into the peritoneum and then the uptake and clearance of the carbohydrate in the blood stream is monitored. This has been done on a variety of established aquaculture species, for example silver perch (Stone et al., 2003a), barramundi (Anderson, 2002), tilapia (Shiau and Chuang, 1995; Anderson, 2002), yellowtail (Masumoto, 2002), carp (Hertz et al., 1989), red sea bream (Koshio, 2002), channel catfish (Wilson and Poe, 1987), white sturgeon (Deng et al., 2001), rainbow trout (Brauge et al., 1994), gilthead seabream (Peres et al., 1999) and European seabass (Peres et al., 1999). In general, fish of a low trophic level tend to be more efficient in the uptake and clearance of glucose compared to carnivorous species (Furuichi & Yone, 1981; Garcia-Riera & Hemre, 1996; Peres et al., 1999).

The species investigated in this study was mulloway (*Argyrosomus japonicus*), a large, estuarine sciaenid of recreational and commercial importance in Australia and South Africa (Fielder & Bardsley, 1999). It's widely distributed including the temperate waters of the African southeast coast, the entire southern seaboard of Australia, off Pakistan and the northwest coast of India in the northern Indian Ocean and in the Northern Pacific from Hong Kong, along the Chinese coast, up to Japan and Korea (Griffiths and Heemstra, 1995). Mulloway are a carnivorous marine species that has had very little research on its nutritional requirements. Target prey of estuarine juvenile mulloway includes teleosts, calanoid copepods, mysids, insects, amphipods and prawns. Coastal juvenile mulloway feed on a wide variety of organisms including benthic, epibenthic and pelagic crustaceans, cephalopods and teleosts (Griffiths, 1997b). A marked shift from prawns to fish with increase in size has also been noted in estuarine mulloway (Fielder et al., 1999).

Mulloway could be a very profitable aquaculture species in Australia. At Sydney Fish Markets in 2003 mulloway on average were sold for \$11.50/kg for wild caught and \$7.05 for aquaculture produced (Sydney Fish Markets Website, 2004). In New South Wales, commercial catches of mulloway have declined over recent years following the introduction of legal size limits to protect fish stocks. Commercial catches which equalled 154t in 1992/93 dropped to 88t (value \$640,000) in 1997/98 (Fielder et al., 1999). Mulloway are a euryhaline, robust species that is both highly fecund and grows quickly. Trials assessing both intensive and extensive rearing techniques show the larvae to be relatively easy to rear (Fielder et al., 1999). As adults, mulloway have many advantageous traits for growout in sea cages. They are known to be gregarious and non-territorial compared to trout and salmon, readily form schools and adjust to captivity quickly, which makes inspection for disease relatively easy. If research into the use of inland groundwater ponds for marine species proves successful, a new industry may develop relatively quickly (ABARE, 2003). The aquaculture production of mulloway in NSW in 2001/02 was reported in the NSW Fisheries Production Report to stand at 500 kg with 25 farms having permits to farm mulloway (NSW Fisheries, 2002).

Due to variations in the protein utilisation and nutritional requirements of fish species, the formulation of cost-effective diets must be done on a species-specific basis. Currently, no data exists on the nutrient utilization and growth of mulloway. The focus of this study is to assess common alternative feed ingredients and their utilization by juvenile mulloway. These were addressed using a variety of methods, including;

1. Evaluating the digestibility of some practical feed ingredients of animal origin, including fish meal, meat meal and poultry meals in the diet of juvenile mulloway

2. Measuring of feed intake and growth of mulloway in the experimental tanks to allow calculations of growth rates. The inclusion of a commercial diet will allow the assessment of growth rates on a nutritionally balanced diet

3. Assessing the mulloway's ability to use dietary carbohydrate through a glucose tolerance test

The formulation of a cost-effective diet and feeding regime is one of the first steps in the development of a successful and profitable mulloway production industry.

MATERIALS AND METHODS

Two experiments were conducted. The first was designed to determine the apparent digestibility of protein and energy from fish meal, poultry meal and meat meal. The second experiment was designed to investigate the carbohydrate utilisation of juvenile mulloway using a classic glucose tolerance trial (GTT).

Experiment 1 – Digestibility of selected feed ingredients

Diets

A basal reference diet was formulated with 49.3% steam dried fish meal (Pesquera Itata, SA, Chile), 49.3% extruded wheat and 1.5% full vitamin and mineral premix (CCD animal health, July 2003) on a dry weight basis. All experimental diets for the digestibility study were composed of 69.8% reference diet and 29.8% test ingredient on a dry weight basis. This 70:30 reference diet: test ingredient ratio was used to ensure palatability of the test diets and limit extreme variations in dietary protein content, whilst supplying adequate levels of the three animal meals to make them testable (Gaylord and Gaitlin, 1996). The test ingredients included steam dried fish meal, meat meal (Ridley Corporation Pty Ltd, Epping, NSW) and poultry offal meal (Steggles/Burten) (table 5).

Chromic oxide was used as an inert marker and incorporated into the diets at 0.5% inclusion level. All ingredients and the reference diet were ground using a hammer mill with a 1.6 mm screen and then thoroughly mixed (Hobart mixer: Troy, City OH, USA). Approximately 400-450 ml distilled water per kg dry mix was added to the dry mix prior to being pelleted through a meat mincer with a 4mm die (Barnco Australia, NSW, Australia). Pellets were then dried in convection driers for $4\frac{1}{2}$ hours until moisture contents were less than 10%. The commercial barramundi diet from Ridley Corporation Pty Ltd used in the experiment also incorporated 0.5% chromic oxide and was ground and pellet in the same way as the experimental diets (Table 5).

Digestibility of selected ingredients and growth

Juvenile mulloway were obtained from Silver Beach Hatchery and transported to NSW Fisheries Port Stephens Fisheries Institute (PSFI) by road. Prior to the experiment, fish were held in recirculation tanks and fed on a commercial barramundi diet from Ridley Corporation Pty Ltd (Epping, NSW). To free fish of ectoparasites, they were treated in 200 mg L-1 formalin for one hour and then flushed through 24 hours before stocking. On the day of stocking, fish were sedated in the recirculation tank

TABLE 5

	Moisture %	Ash %	Protein %	Energy KJ gm	Phosphorous %	Chromic Oxide %
Ingredients						
Fish meal	6.6	14.9	77.3	22.9	-	-
Meat meal	3.3	32.7	56	17.6	-	-
Poultry meal	5.1	9.2	75.3	23.7	-	-
Diets						
Commercial	5.0	13.3	61.1	20.0	2.7	0.3
Reference	8.3	9.7	39.7	24.3	1.7	0.3
Fish meal	7.4	11.3	57.5	20.1	2.0	0.3
Meat meal	6.8	16.4	51.1	20.9	3.3	0.3
Poultry meal	6.9	9.6	57.9	30.9	1.7	0.3

Dry basis proximate composition and gross energy content of test ingredients and diets

with 10 mg L^{-1} benzocaine solution. Sixty fish were then randomly selected from the population and anaesthetised further in 20 mg L-1 benzocaine solution. These fish were weighed provided a target weight range, 75-100 grams, that encompassed approximately 50% of the population. Within this weight range a total of 150 fish were then selected, weighed and their fork length measured before being systematically assigned to fifteen experiment tanks.

Once in the laboratory, fish were fed the experimental diets containing chromic oxide. Following a week acclimation period faecal samples were pooled for each tank for until sufficient sample was collected (2 weeks). At the end of the experiment the fish were anesthetised dissected and their digestive morphology examined. The intestine was cut out and measured (not stretched) along with the weight and total length of each fish.

Fish were weighed and measured at the beginning and end of the digestibility experiment to give growth data for a period of 21 days. This data was used to calculate specific growth rate of mulloway for each of the diets. Specific growth rate (SGR) was calculated as:

SGR (% day⁻¹) = $(\ln(W_{W1}) - \ln(W_{W0})/T*100$

where W1 is the fish weight at the end of the study, W0 the fish weight at the beginning of the study; and T the time in number of days.

Laboratory facility

Diet treatments were assigned to fifteen 170 L cylindro-conical digestibility tanks using a random number generator (Figure 6), with three replicate tanks for each of the five dietary treatments. Photoperiod was set for a 12 hour light/dark cycle beginning the light phase at 0600 h.

Fish were fed their diets at 0830 h for 3 hours every morning using a spring operated, conveyor belt feeder. After feeding, uneaten feed was collected and tanks were cleaned thoroughly to remove accumulated waste. Faeces were allowed to settle overnight (approximately 18 hours) and were collected each morning prior to feeding, dried under vacuum at room temperature for 24 h (silica

gel) and then frozen (less than -15°C). Faecal samples from day 9 were collected pooled until sufficient sample for chemical analysis was obtained.

Water used in the laboratory was evaluated regularly to maintain the desired temperature (25-26°C), treated for pathogens and subjected to continuous unidirectional flow and filtration through sand and diatomaceous earth filters. The water was supplied to the experimental tanks at a flow rate of approximately 1 L min⁻¹. Effluent water was drained from the experimental tanks via standpipes and 20-25% was discarded. The remainder was collected in common sump and recirculated through a biological filter for reuse in the laboratory. Oxygen was supplied both to water in holding tanks and through 2 air stone infusers in each digestibility tank. Air supply was regulated to maintain dissolved oxygen of 4-6 mg L⁻¹ and pH between 6.8-8.0. Total ammonia-nitrogen was measured weekly and did not exceed 0.4 mg L⁻¹.



FIGURE 6

Experiment tanks used to hold mulloway for digestibility experiment

Chemical analyses

Dry matter for the feed and faeces was determined by drying samples (2 g) in an oven at 105°C till a constant weight was attained (16 h). The loss in weight on drying was recorded as the moisture content of the sample (AOAC; method 934.01, 2000). Energy was determined by bomb calorimetry using a Gallenkamp ballistic bomb calorimeter. All dried sub samples were ground, weighed and combusted in the bomb calorimeter, using benzoic acid as a standard. Nitrogen was measured volumetrically following combustion using a Leco-CNS analyser. Nitrogen values were multiplied by 6.25 to estimate crude protein. Phosphorous was analysed using atomic absorption spectrometry. Sub samples were ashed (550°C for 12 hours) and digested in a solution containing 2% nitric acid and 2g L⁻¹ potassium chloride. These solutions were then analysed on an ICP atomic absorption spectrometer (Mambrini and Peyraud, 1994). Chromic oxide content of diet and faecal samples was outsourced to the Food and Agricultural Laboratories Australia Pty Ltd (FALA, Qld, Australia) for analysis using ICP atomic spectrometry.

Digestibility of the diets was calculated by reference to the dry matter nutrient or energy content of diets and faecal samples and their corresponding concentrations of chromium. The apparent digestibility coefficients (ADC) for the reference and test diets were calculated using the formula:

Diet ADC (%) = 100 x $[1 - (F_{Nut}/D_{Nut} x D_{Cr}/F_{Cr}),$

where $F_{Nut} = \%$ nutrient or energy in faeces; $D_{Nut} = \%$ nutrient or energy in diet; $D_{Cr} = \%$ chromic oxide in diet and $F_{Cr} = \%$ chromic oxide in faeces (Cho and Kaushik, 1990).

Apparent digestibility coefficients of the individual test ingredients were determined after considering the difference between the nutrient or energy content of the reference and test diets. The following formula was applied:

 AD_{ING} (%) = [(Nut_{TD} x AD_{TD}) - (0.69 x Nut_{RD} x AD_{RD})] / (0.29 x Nut_{ING})

where AD_{ING} is apparent digestibility of nutrient or energy in the test ingredient; Nut_{TD} is the nutrient or energy concentration in the test diet; AD_{TD} is the apparent digestibility of the nutrient or energy in the test diet; Nut_{RD} is the nutrient or energy concentration in the reference diet; AD_{RD} is the apparent digestibility of the nutrient or energy in the reference diet and Nut_{ING} is the nutrient or energy concentration in the test ingredient (Sugiura et al., 1998).

Statistical analyses

All response data were tested for heterogeneity of variances (Cochran's test) before conducting oneway ANOVA to compare treatment means. Where they occurred, statistical differences between treatment means were separated using Tukey's test. Statistical analyses were performed using Minitab (Version 13.1).

Experiment 2 – Acute glucose tolerance test

This experiment was designed to investigate the 72 hour response of juvenile mulloway to an intraperitoneal injection of carbohydrate at a dose rate of 1 g D-glucose kg⁻¹ body weight. The experiment was designed to include 1 major factor of interest (i.e. glucose injection) and 2 procedural controls; sham injection or handling effect. The procedural controls were included because previous research has shown that cortisol response can adversely affect blood plasma glucose levels in fish. As such, confounding issues related to stress must be taken into consideration when designing glucose tolerance experiments (Stone et al., 2003a). Therefore, juvenile mulloway were given a) an injection of 1 g D-glucose kg⁻¹ body weight or b) handled such that the fish was subjected to the same handling procedure as the glucose group, but not injected or c) exposed to the same handling procedures as the glucose group but received a sham injection of sterile isotonic saline solution (0.9% NaCl). Four consecutive GTT trials were run to in order to obtain 4 independently collected replicates for each of the 3 treatment effects over time.

Stock solutions and injection procedures

A stock glucose solution was prepared by mixing 40 g of analytical grade D-glucose powder per 100 mL sterile saline solution to give a 2 g glucose per 100 mL standard solution. Individual dose rates were determined by weighing each fish and calculating the desired volume of solution (i.e. either glucose or saline) using a constant ratio of stock solution to fish weight. Intra-peritoneal injections were made with 1 mL syringes fitted with 27 gauge hypodermic needles. After injection or handling procedures the exact time was recorded and the fish was transferred to a separate holding cage to await collection of blood. The holding cages consisted of a 200 L holding tub with a plastic cage inside that allowed us to quickly remove the fish from the tub without handling stress (Figure 7).



FIGURE 7

Experiment tanks used for acute glucose tolerance test.

Fish

Approximately 120 Juvenile mulloway were obtained from O'Donohue Filter Sand and Gravel Pty Ltd's mulloway hatchery (Millers Creek, NSW, Australia) and transported by road to PSFI. Before the experiment they were held in a 10 000 L recirculation tank with a flow through rate of 3 L min⁻¹. Water temperature was maintained at $21 \pm 2^{\circ}$ C using 2 KW immersion heaters. The fish were fed a 6 mm commercial sinking diet (snapper diet, Ridley Corporation Pty Ltd, Epping, NSW, Australia) twice daily for 2 weeks prior to commencing the experiment.

One week before the experiment commenced, fish (weight range 86-288 g) were size graded into 4 trial groups and each group was placed in a separate, black-lined perforated cage (approximately 200 L). Each cage contained approximately 26 fish. At the beginning of each trial, one randomly selected cage of fish was anaesthetised in a 150 L tank using a starting dose 20 mg benzocaine L^{-1} . These fish were then transferred into a holding bin containing 10 mg benzocaine L^{-1} and an air stone diffuser. Individual fish were then selected from this tank and randomly assigned to one of the 3 experimental treatments.

Blood sampling

At the beginning of each trial, 3 anaesthetised fish were selected at random and blood samples taken immediately (Figure 8). These samples were later randomly assigned to each of the three treatments and used as the initial samples to provide basal blood plasma glucose levels for each trial (i.e. T_0). Blood samples were taken from the fish at 1, 3, 6, 12, 24, 48 or 72 hours following treatment. An attempt was made to sample blood from each fish within 1 minute to reduce the confounding effects of stress responses. Occasionally blood samples could not be obtained from every fish. Facility designs and time limitations dictated that including enough spare fish was not always possible. For this reason any samples missed during a trial run were collected using extra samples included in subsequent trial runs in order to provide an orthogonal data set. Following blood sampling fish from each trial were recovered and returned to their original holding cage. All fish in this experiment were handled and sampled only once.



FIGURE 8

Sampling blood from juvenile mulloway

Water quality was assessed before each trial to maintain a temperature of $21 \pm 0.12^{\circ}$ C, pH of 7.7 ± 0.05 and salinity of $2.9 \pm 0.02\%$. Oxygen was supplied through an air stone infuser in each cage and air supply was regulated to maintain dissolved oxygen of 8.8 ± 0.04 mg L⁻¹.

Chemical analysis

Blood samples were analysed for plasma glucose by the Hunter Area Pathology Service (HAPS; John Hunter Hospital, Newcastle; NATA accredited) using the enzymatic reference method with hexokinase. Hexokinase (HK) is used to catalyse the phosphorylation of glucose by ATP to form glucose-6-phopshate and ADP. Following this reaction a second enzyme, glucose-6-phopshate dehydrogenase (G6PDH) is used to catalyse the oxidation of glucose-6-phospate by NAD- to form NADH. The concentration of NADH formed is determined by measuring the increase in absorbance at 340 nm and is directly proportional to the glucose concentration (Roche Diagnostics, 1998).

Statistical analysis

Statistical analysis was done using two-factor ANOVA with treatment type [glucose injection; sham injection; handling control] as the first fixed factor and sampling time as the second fixed factor using GMAV (Institute of Marine Ecology, Sydney University, 1997).

RESULTS

Digestibility of diets and ingredients

The compositions of the three meals were quite high in protein (all >55%). Gross energy content ranged from 17.7 MJ kg⁻¹ for meat meal to 23.7 MJ kg⁻¹ for poultry meal. For the diets, the commercial diet and fish meal diet were higher in protein content than the other three diets. Poultry meal was the highest in energy content and the lowest in ash content. Meat meal was highest in ash content (16.4%) (Table 5).

For organic matter, fish meal and meat meal both recorded high values (110% and 104% respectively), with poultry meal the only ingredient scoring 71%. The protein in all the ingredients was well digested with poultry meal the lowest at 84%. For energy fish meal and poultry meal both recorded values around 80%, with meat meal slightly lower at 68% (Table 6).

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For organic matter all diets scored digestibility values >60%, with the commercial Ridley's barramundi diet scoring the highest (81%) followed by the fish meal diet (71%), meat meal diet (67%), poultry meal diet (60%) and the reference diet (58%). All diets had highly digestible protein values (all >85%), with fish meal being the most digestible (93%). For energy, all diets recorded digestibility values >80%, with the commercial diet (93%) scoring the highest and the meat meal diet (80%) the lowest (Table 6).

TABLE6

Mean percent (\pm SEM, n = 3) apparent digestibility coefficients for organic matter, protein and gross energy of the test ingredients and diets with phosphorous also for the diets. Fish meal, meat meal and poultry meal diets were composed of 70% reference diet and 30% test ingredient. Significant different values are labelled with a different letter (Tukey's test).

	% Organic matter	% Protein	% Energy	% Phosphorous
Ingredients				
Fish meal	$110.0 \pm 4.9^{a}_{a}$	100.1 ± 2.8	82.8 ± 6.6	-
Meat meal	103.6 ± 3.5	92.8 ± 4.2	67.7 ± 4.5	-
Poultry meal	70.7 ± 8.2	84.0 ± 2.2	79.1 ± 5.5	-
Diets				
Commercial	80.9 ± 1.0^{a}	85.9 ± 1.5^{a}	93.1 ± 0.9^{a}	39.3 ± 1.7^{a}
Reference	57.6 ± 3.1^{b}	90.7 ± 1.4^{ab}	86.2 ± 1.0^{ab}	60.1 ± 2.5^{b}
Fish meal	$71.0 \pm 2.7^{\circ}$	92.8 ± 1.2^{b}	83.6 ± 1.8^{b}	58.5 ± 2.7^{b}
Meat meal	$67.3 \pm 1.7^{\circ}$	89.5 ± 1.5^{ab}	80.4 ± 1.0^{b}	40.1 ± 2.6^{a}
Poultry meal	60.3 ± 4.8^{bc}	85.8 ± 0.9^{a}	82.5 ± 1.6^{b}	60.5 ± 1.4^{b}

Statistically, fish meal and meat meal were different to poultry meal only for organic matter (P<0.05) (Table 7). Energy was not statistically different for ingredients. All variables, dry matter, protein, energy and phosphorous were statistically different for diets (Table 8).

TABLE 7

One-way analysis of variance table for dry matter, protein and energy with ingredient as the fixed factor.

	Source	DF	SS		MS	F	Р
Dry matter							
	Ingredient		2	2261	1330	9.65	5 0.013
	Error		6	827	138		
	Total		8	3488			
Protein							
	Ingredient		2	388.7	194.30	4.85	5 0.056
	Error		6	240.4	40.10		
	Total		8	629.1			
Energy							
	Ingredient		2	373	187	1.49	0.298
	Error		6	751	125		
	Total		8	1124			

TABLE 8

One-way analysis of variance for dry matter, protein, energy and phosphorous with diet as the fixed factor.

	Source	DF	SS	MS	F	Р
Dry matter						
	Diet	4	1025.81	256.45	29.59	0.000
	Error	10	86.66	8.67		
	Total	14	1112.47			
Protein						
	Diet	4	112.56	28.14	4.01	0.034
	Error	10	70.19	7.02		
	Total	14	182.75			
Energy						
	Diet	4	292.10	73.03	10.57	0.001
	Error	10	69.10	6.91		
	Total	14	361.20			
Phosphorous						
	Diet	4	1445.50	361.40	18.34	0.000
	Error	10	197.00	19.70		
	Total	14	1642.5			

There was no significant difference found for the specific growth rates, but a general trend was found that supported the other data, showing that fish meal and meat meal performed slightly better than the other diets across the 21 day period (Figure 9).



FIGURE 9

Specific growth rate for each diet over the 21 day period. Data are means \pm SE (n=3). Bars are not significantly different (P>0.05).



Intestinal Morphology

FIGURE 10

Intestinal length (IL) vs. body length (BL) giving the regression equation IL = 0.92BL + 18.4 (R2 = 0.21, n=110) for juvenile mulloway. Other species regression equations plotted on graph, including omnivorous/detritivorous Tilapia (Popma and Masser, 1999), omnivorous (emphasising animal feed sources) Japanese catfish (Yada and Furukawa, 1999), carnivorous Rainbow trout (Smith, 1978), omnivorous fathead minnow (IOWA DNR, 2004) and omnivorous (emphasising plant food sources) carp (Smith, 1978).

The intestinal length for the juvenile mulloway was approximately 0.9 times the body length of the individual ($R^2 = 0.21$, n = 110) (Figure 10).

Acute glucose tolerance trial

Blood plasma glucose levels peaked at approximately 23 mmol L^{-1} in the glucose injected fish between 3 and 6 h after treatment (Figure 11). Blood plasma glucose levels remained elevated up to 48 h after injection with D-glucose but had returned to baseline levels by 72 h (Figure 11). The blood plasma glucose concentrations after 1, 3, 6, 12 and 24 h differed significantly (P<0.05) from the baseline levels and those recorded after 48 and 72 hours. The 'sham' and handling treatments also experienced a slight increase in blood plasma glucose levels between 1 and 3 h after treatment. These were not found to be statistically different (P>0.05) from the recorded baseline levels. Both 'sham' and 'handling' treatments were found to be statistically different (P<0.05) from the glucose treated fish between 1 and 24 h after injection (figure 11).



FIGURE 11

The average blood glucose concentration for juvenile mulloway (mmol L-1) after handling or injection with sterile saline solution or glucose at 1g kg-1 body weight over 72 h.

DISCUSSION

The protein and nutrient requirements for fish differ and for this reason nutritional research must be species specific. To date no research has been published on the nutritional requirements of mulloway. Other sciaenoids, such as red drum (*Sciaenops ocellatus*), orangemouth corvina (*Cynoscion xanthulus*) and white seabass (*Atractoscion nobilis*) have been extensively researched for aquaculture purposes. Studies have shown that there are strong similarities between the life history and breeding requirements of mulloway and red drum.

Some digestibility coefficients >100% were recorded. These were more than likely due to errors in measurement, which are magnified in the calculations for the digestibility coefficients, possible interactions between nutrients in the reference diet and test ingredients or differential leaching of some nutrients.

The reasons behind formulating aquafeeds with large quantities of fish meal are apparent when digestibility values for dry matter, protein and energy are considered. The high digestibility coefficients recorded here for mulloway fed a fish meal based diet are consistent with previous research with other species, such as silver perch (Allan et al., 2000b), red drum (Gaylord and Gaitlin, 1996; McGoogan and Reigh, 1996), salmonoids (Cho and Kaushik, 1990; Hajen et al., 1993; Sugiura et al., 1998), rockfish (Lee, 2002), yellowtail (Masumoto et al., 1996), tilapia (Hanley, 1987) and channel catfish (Wilson, 1991).

Mulloway were capable of digesting the protein in all the ingredients tested. However, the digestibility for meat and poultry meal was slightly lower than fish meal. It has been reported that the main disadvantage to using well-digested, high protein alternative ingredients is that the essential amino acid profile and availability are slightly inferior to that of fish meal. Animal meals are generally good sources of lysine, the first limiting amino acid in fish feeds, but poor sources of the sulphur amino acids methionine and cystine, which are needed for protein synthesis and other physiological functions in fish (Hardy and Barrows, 2002). Whilst both meat meal and poultry meal digestibility coefficients recorded were >80%, protein digestibility tends to reflect amino acid availability, which can affect ingredient utilization by fish (Allan et al., 2000b). Meat meal performed better than poultry meal for dry matter and protein digestibility, whilst the energy in poultry meal was higher than in meat meal. Red drum has been found to utilise fish meal very efficiently and grow faster on fish meal than on any other protein source. They were also found to digest the protein, lipid and energy of other animal feedstuffs very well, which can be attributed to its carnivorous nature (Moon and Gaitlin, 1994; Gaylord and Gaitlin, 1996; McGoogan and Reigh, 1996; Kureshy et al., 2000).

Performance studies which measure various growth parameters are used to evaluate and compare the use of different fish diets. These experiments are usually conducted for a longer period of time than our digestibility experiment, but we recorded the growth of the fish to indicate which diets were best for mulloway. None of the diets were statistically different (P>0.05) from each other but these results must be treated with caution, as the experiment was not conducted as a growth experiment or for a reasonable period of time. A general trend showing fish meal and meat meal diets to perform well did appear, but as mentioned was not statistically different from the other three diets (P>0.05).

Growth trials with the closely related red drum showed that increasing fish meal content in test diets resulted in increases in weight gain, survival and feed efficiency ratios. In diets formulated with low levels of fish meal, methionine supplementation was required to support high growth rates. Based on these results, a low fish meal diet incorporating palatability enhancers was developed, resulting in weight gain and feed efficiency ratios not significantly different from those obtained with a high fish meal diet (Davis et al., 1995).

The factors that affect digestibility are important to consider when determining nutrient availability and formulating artificial feeds. The feeding habits and natural diets of different species of fish influence their type of digestive system. The digestive tract anatomy ensures that the feed intake, digestion and absorption are optimised. Typically carnivorous species have specialised prey capture mechanisms and as their food is generally highly digestible, breakdown begins as soon as the food reaches the simple stomach. The stomach tends to be large and muscular to accommodate a single large meal and the gastrointestinal tract is generally thick and short (Wee, 1992). The intestinal hydrolytic capacity and the gut transit time are believed to be important factors affecting the digestion and breakdown of carbohydrates by fish (Deng et al., 2001). The gastrointestinal tract morphology has been examined in many species and it has been noted that the intestinal length tends to be short in carnivorous fish, longer in omnivorous fish and longest in herbivorous and detritivorous fish (Figure 12). Gut lengths are usually greater than one times the body length in carnivore, such as rainbow trout (approximately 0.7 times the body length) (Smith, 1978), and more than twenty times the body length in certain plant and detritus-eating species (Horn, 1998). There are exceptions to this generalisation, such as herbivores like the stomachless *Odax pullus* with a relative gut length of 1.5 (NRC, 1997 in Horn, 1998) and *Hyporhamphus melanochir* of 0.5 (Robertson and Klumpp, 1983 in Horn, 1998).

In studies on three coral reef families (*Labridae*, *Chaetonidae* and *Pomacentridae*) surveyed in the Great Barrier Reef intestinal length was most strongly attributable to diet.



FIGURE 12

Diagrammatic representation of the digestive systems of four fish described in the text, arranged in order of increasing gut length. a. Rainbow trout (carnivore);b. Catfish (omnivore emphasizing animal sources food); c. Carp (omnivore, emphasizing plant sources of food); d. Milkfish (microphagous planktovore). (Smith, 1978)

Morphological variation was evident within the families with corallivores possessing the longest intestines, followed by herbivores, omnivores and carnivores. However, it was also noted that the magnitude of this variation differed greatly for each family showing an interaction between diet and phylogenetic factors. As such it was suggested that while direct comparisons of relative intestinal length between unrelated taxa may be a relatively reliable indicator, major phylogenetic differences between taxa have the potential to confound these analyses (Elliot and Bellwood, 2003).

The mulloway intestinal length was on average only 0.9 of the total body length ($R^2 = 0.21$) (Figure 11). The basis for this intestinal length and trophic level ratio seems to be that animals on low protein, high roughage diets require longer guts in order to process the large amounts of poor quality feed. In contrast, the nutrients of carnivorous diets tends to be quickly and easily digested and absorbed which

do not need the extensive amount of surface area necessary in the intestines of some plant and detritus eating fish (Horn, 1998).

Fish glucose tolerance and carbohydrate use literature has emphasized the confounding effects of primary stress responses in fish to experimental procedures such as handling, injection, repeated disturbance if sampling from the same tank and blood sampling. This issue must be adequately addressed during glucose tolerance tests as elevated plasma glucose due to stress has been found in numerous studies to easily confound results (Robertson et al., 1987; Robertson et al., 1988; Stone et al., 2003a). To confirm that handling and injection procedures did not significantly influence plasma glucose concentrations two controls were included in the experiment, a handling procedural control and a 'sham' injection control. There was a slight increase in plasma glucose for both the handling and sham control fish when compared to the initial basal levels. However, these were not statistically significant and were small compared with the elevation in plasma glucose levels in the fish receiving an injection with glucose (figure 12). The rapid initial glucose rise that occurs after exposure to stressors is due mainly to the increased catecholamine secretion, whilst the sustained hypoglycaemia is thought to be attributable to cortisol (Robertson et al., 1988).

Mulloway given an intra-peritoneal injection of 1 g glucose kg^{-1} body weight were not as tolerant of glucose as other species that have previously been exposed to the same procedures. The plasma glucose of mulloway was found to peak 1 to 3 hours after first injection and to stay elevated for 24 hours after initial injection. Mulloway plasma glucose stayed significantly elevated much longer than that observed in omnivorous silver perch (returned to basal levels within 12 hours) (Stone et al., 2003a), omnivorous/detritivorous tilapia (within 12 hours) (Anderson, 2002) and the carnivorous species of barramundi (within 16 hours) (Anderson, 2002), gilthead seabream (within 24 hours) (Peres et al., 1999) and European seabass (within 24 hours) (Peres et al., 1999) (figure 13). The clearance rate of glucose is believed to be indicative of the absorption efficiency of carbohydrate and the differing abilities have been attributed to the major differences in facilitated glucose transport mechanisms and probable differences in metabolic enzyme activities between species (Stone et al., 2003a). based on this figure mulloway appears to be less capable of absorbing carbohydrate than the other species listed. The most efficient species on this graph are the omnivorous and detritivorous species of silver perch and tilapia. The other carnivorous species, though more tolerant than mulloway, were less capable of absorbing and clearing the injected glucose from their blood (figure 13).



FIGURE 13

Response of six species to intraperitoneal injection of 1g glucose kg-1 body weight. (a) from this study, (b) from Stone et al., 2003a, (c) from Anderson, 2002, (d) from Peres et al., 1999.

Previous research has shown that some carnivorous species that have not performed well in glucose tolerance tests can have good growth rates, increased glucose tolerance, efficient adaptation of hepatic carbohydrate-metabolizing enzymes and show a protein-sparing effect when fed carbohydrate when fed a diet with limited carbohydrate for a long period of time (Shimeno et al., 1979). The protein-sparing effect of carbohydrates or lipids means that these macronutrients are used by the fish as an energy source leaving the protein to be used for growth. Carnivorous yellowtail grew well with up to 20% included carbohydrate (Shimeno et al., 1979), and red drum tolerated up to 35% carbohydrate in their diet without it being detrimental (Gaitlin, 2002).

The general method of carbohydrate digestion is hydrolysis of complex carbohydrates extracellularly in the stomach, intestine, and caeca, with membrane-linked hydrolysis in the anterior intestine and caeca by a variety of carbohydrases. The products of this hydrolysis are simple carbohydrates (polysaccharides and monosaccharides. It is known that in mammals the transportation of monosaccharides from the lumen of the small intestine by an active transport mechanism in the brush border. However it is unclear whether this is also true in fish (Rust, 2002). Differences in carbohydrate digestibility amongst species are in part attributable to the different amounts and types of carbohydrases. The relative utilization of dietary carbohydrate varies and appears to be related to the complexity of the carbohydrate. In general, cooked starch and dextrin are better utilised by fish than simple sugars. The prolonged hyperglycaemia observed in fish following glucose tolerance tests and their relative inability to utilise simple sugars has been attributed to a few factors including low hexokinase activity and a lack of an inducible glucokinase enzyme; glucose not being as potent a stimulus for insulin release as many amino acids; the possible inhibition of insulin by somatostatins released in response to high blood glucose levels; and the relatively low number of insulin receptors in fish compared to in mammals (Wilson, 1994).

Growth trials substituting wheat starch at different inclusion levels could indicate if carbohydrate can have a protein-sparing effect in this species. Ellis and Reigh (1991) found that red drum appeared to have a limited ability to utilise dietary carbohydrate as an energy source. Dietary energy levels and the carbohydrate content were inversely related to weight gain, feed efficiency, apparent net protein retention and apparent net energy retention. A greater protein sparing effect was observed from diets incorporating high levels of dietary lipids.

Previous research into the use of alternative feed ingredients for red drum showed that in general animal products had higher digestible energy coefficients than plant meals. Dietary fibre is not utilized by fish and thus it may cause lower dry matter and energy digestibilities of fish (Lee, 2002). Red drum is not an exception, it appears to be unable to effectively digest the nitrogen-free extract portions of plant products and authors have concluded that high levels of fibre were not desirable in red drum feeds (McGoogan and Reigh, 1994; Gaylord and Gaitlin, 1996; McGoogan and Reigh, 1996).

CONCLUSIONS

The benefits to the mulloway of cheaper, better diets are obvious – reduced growing costs which leads to increases in production. It is difficult to attribute any increases in production to any single factor, but the improvement of diets and feeding have been seen to play a significant part in the advancement of the silver perch and barramundi industries in Australia (Allan et al., 2003) and the channel catfish industry in America (Lovell, 1991). The use of agriculture products in aquafeeds will also increase marketing opportunities for agriculture producers. The demand for aquaculture feed on the international market has been growing rapidly over the past decade, and is expected to continue to rise from the 15 million tonnes required in 2000 to 27 million tonnes in 2010 (Coutteau et al., 2000). Thousands off tonnes of lupins and meat meals are already being sold offshore for use in aquafeed production (Allan et al., 2003).

The development of a successful and cost-effective mulloway industry will not only reduce pressure on diminishing wild stocks, but also help reduce the reliance on fish and fish product imports. Despite

recent advances in aquaculture production imports have increased by 59% between 1992/93 and 2002/3 with 249 000 t of fish and fish products (including 55 788 t of live, fresh or frozen fish or fillets) being imported in 2002/03 (ABARE, 2003). The profitability of an aquaculture enterprise is largely affected by feed costs, which can account for 50-70% of the operating costs. However, the temptation to focus on the cost of feed can be counterproductive if biological evaluation is not considered. Diet formulation and manufacture integrate many sources of information including the nutrient requirements of the species the diet is being produced for, its life stage, any special requirements for feeding and culture, production goals, feed quality and product quality. In this context, the costs of feed can be understood in terms of units of product sold and therefore the optimization of feeds and feeding practices can be achieved (Hardy and Barrows, 2002).

This study shows that meat meal is a highly potential substitute for fish meal in diets formulated for mulloway. However, high digestibility does not necessarily mean that a product will be well utilised. All three ingredients tested for digestibility had reasonably high energy and protein digestibilities and therefore evaluation through a growth assay would be beneficial to determine utilisation by juvenile mulloway. These studies would also reveal any issues concerning palatability or antinutritional factors (especially if including any plant products) that could affect the effectiveness of a diet. Mulloway's apparent inability to cope with carbohydrates could limit the inclusion of plant meals in this species diet. However, in closely related species, inclusion of a limited amount of plant material has been found not to be detrimental to the health or growth of the fish (Gaitlin, 2002). Therefore the evaluation of mulloway's utilisation of a range of feed products would be the logical next step. The information accumulated so far is fairly consistent with that for red drum, which also digest animal meals well and a fairly intolerant to carbohydrate (Gaitlin, 2002). The advancement in mulloway nutrition knowledge can be used to help the industry develop into a successful and sustainable aquaculture asset.
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APPENDIX

Statistical Analysis

1. One-way ANOVA for Growth Parameters

	Source	DF	SS	MS	F	Р
% Weight Gain per day	V					
	Diet	4	0.143	0.0358	1.12	0.401
	Error	10	0.3197	0.032		
	Total	14	0.4628			
Specific Growth Rate						
	Ingredient	4	0.0972	0.0243	1.14	0.392
	Error	10	0.2131	0.0213		
	Total	14	0.3103			

2. Two factor ANOVA table for acute glucose tolerance test.

Source	DF	SS	MS	F	Р
Treatment	2	32.616	16.308	90.79	0.000
Time	7	10.449	1.493	8.31	0.000
Treatment x Time	14	17.499	1.250	6.96	0.000
Error	72	12.933	0.180		
Total	95	73.497			

9.5 A pilot study investigating the post-prandial glycaemic response of yellowtail kingfish *Seriola lalandi* fed high dietary levels of pregelatinised wheat starch or D-glucose

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1. INTRODUCTION

Glucose tolerance tests (GTT) have been widely used to study the ability or inability of different fish species to deal with carbohydrates (CHO). More often and because it is difficult to feed fish diets that contain elevated levels of CHO, glucose is injected into the fish and then changes in concentration of blood or plasma glucose is monitored over some time period. In general, direct injection of highly available CHO's such as glucose significantly elevate the circulating levels of glucose and induce a prolonged state of hyperglycemia which indicates an intolerance of CHO. This response is seen in many fish species, especially the carnivores. However, this response is often different when CHO's are fed to fish, with many species showing some ability to utilise CHO when it is included in the diet (Peres & Olivia-Teles 2002; Stone *et* al 2003; Wu et al. 2007). The ability of fish to utilise dietary CHO has been found to be strongly dependent on ingredient processing, with more complex starches often found to be better utilised than simple monosaccharide's such as glucose. This increased utilisation is most likely due to the slow regulated release of glucose from more complex forms into the blood stream due to a prolongation of the digestive process as opposed to what occurs when pure glucose is included directly in the diet.

This pilot study was designed to provide information that would assist in the planning and design of future experiments assessing the ability of yellowtail kingfish *Seriola lalandi* to utilise CHO, specifically how they deal with diets containing high concentrations of starch or one of its digestive breakdown products, glucose. The ability of yellowtail kingfish to cope and regulate the uptake of glucose from these diets was evaluated by monitoring changes in post-prandial plasma glucose concentrations over a 48 hour period.

The specific aims of this pilot study were to: -

- Determine the 48 h post-prandial glycaemic response of yellowtail kingfish fed a diet containing 40% gelatinised wheat starch, 40% D-glucose or a control diet containing low levels of carbohydrate
- Identify major peaks in plasma glucose and duration of response
- Contrast differences in intensity and length of hyperglycaemia following intake of a simple (D-glucose) versus complex carbohydrate source (gelatinised wheat starch).
- Determine the willingness and amount of feed yellowtail kingfish would consume after being starved for a period of 48 h and stocked individually into 200 L cages

2. MATERIALS AND METHODS

2.1 Experimental diets

Three test diets were prepared. One of the diets contained 40% by weight of 100% pre-gelatinised wheat starch and the second diet contained 40% by weight of D-glucose (D-(+)- glucose 99.5%; SIGMA-Adlrich Pty Ltd, Castle Hill NSW Australia). The third diet was designed to be low in carbohydrate and acted as a procedural control. Each of the diets was based on a mix of protein sources including fish meal, meat meal, poultry meal and dehulled lupins. Fish oil was included in each diet as was a vitamin/mineral premix (DSM Nutritional Products, Wagga Wagga, NSW,

Australia). The ingredient composition of diets is presented in Table 1. Prior to mixing, all coarse ingredients were ground in a laboratory scale hammer mill fitted with 1.5 mm screen (C-E Raymond Inc. IL, USA). Ground ingredients were combined on a dry weight basis as per Table 1 and mixed dry prior to the addition of fish oil (Hobart Mixer, Hobart Corporation, Troy, OH, USA). Distilled water was then added to each batch to form a moist dough suitable for cold press pelleting. Diets were pressed into pellets using a small scale meat-mincer fitted with a 6mm die plate (Barnco Australia Pty. Ltd., Leichardt, NSW, Australia). Afterwards, wet pellets were dried in a convection dryer at low temperature (<40 °C) for approximately 7 hours. After drying all diets were frozen at < -15° C.

2.2 Experimental fish and facilities

This study used juvenile yellowtail kingfish (mean weight \pm SD = 262.7 \pm 53.6 g; n=56) which were progeny of wild-caught brood stock held at Industry and Investment NSW Port Stephens Fisheries Institute, Australia. Prior to the study, fish were housed in a 10 kL tank and fed a high protein, low carbohydrate commercial marine finfish diet (Skretting Australia Pty. Ltd, Cambridge, Tasmania; crude protein 50%, crude lipid 17%, gross energy 21 MJ kg⁻¹).

The pilot study was performed in 3 x 10 kL tanks housing 11 x 200 L cylindrical floating cages. Each 10 kL tank was filled with clean disinfected estuarine water that was heated to 20°C. Tank water was not exchanged during the study however each 10 kL tank was aerated via a large central air-stone diffuser to ensure adequate oxygenation (DO₂ > 6.0 ppm). Experiment cages were spaced evenly around the perimeter of the larger holding tanks and secured in place. Experiment cages were constructed of 9mm plastic mesh and were lined with black vinyl to reduce internal and external disturbance of fish. Each cage was fitted with a lid to prevent inadvertent escape of fish.

2.3 *Experimental procedure and collection of blood*

Prior to the experiment, small groups (33) of previously size graded fish were held in 200 L floating cages secured around the perimeter of a 10 kL holding tank. Individual fish were then transferred directly from the holding cage into one of 33 experiment cages. Fish were subsequently fasted for 48 h prior to the test diets being offered. Prior to feeding individual fish were randomly allocated to one of the 3 test diets (procedural control, 40% pre-gelatinised starch or 40% glucose) and one of 11 post feeding sampling times (0, 1, 2, 3, 6, 9, 12, 18, 24, 36 or 48h). Fish were then offered their allocated diet and carefully fed to apparent satiation. The exact time of feeding was recorded for each cage to ensure post-prandial sampling time was accurate. During the feeding period, the blood of 3 unfed fish (0 h) was collected in order to establish a base-line plasma glucose concentration for each run.

All fish were captured without anaesthetic and their blood was withdrawn directly from the caudal vein. In addition, all blood samples were taken within 1 minute of capture to ensure that stress-induced changes in blood glucose concentration did not occur (Stone et al. 2003; Booth et al. 2006). Blood was sampled from each fish only once after which fish were returned to a separate holding tank to recover. During the withdrawal of blood, fish were captured from their cage and placed in the cavity of a soft-foam block covered with wetted plastic so that the ventral surface of the fish was exposed. Blood was then withdrawn from the caudal vein (< 1mL) using a 23 gauge x 1.25 mm hypodermic needle fitted to a 3ml syringe (Becton-Dickinson B-D, Singapore). The procedure was repeated to provide duplicate replicates for each sample time x diet treatment.

2.4 Glucose determination

Blood samples were immediately analysed for plasma glucose (mM) using a calibrated Accu-chek performa® hand-held blood glucose meter (Roche Diagnostics Australia, Castle Hill, NSW, Australia). The Accu-chek Performa® uses an enzymatic reaction whereby the enzyme (dehydrogenase) on each test strip in the presence of the co-enzyme Pyrroloquinoline quinone (PQQ) creates a small DC electrical current when reacting with the amount of glucose in each blood sample (Roche Diagnostics 2006). The meter is then able to interpret the strength of this electrical current and

convert it directly to a plasma glucose reading (Roche Diagnostics 2006). Prior to the study the glucose meter was calibrated to a single batch of test strips according to the manufacturer's instructions. One test strip was used for each fish.

2.5 Water quality / controlled parameters

During the experiment, pH (7-8), salinity (29.8-30.1 ppt), temperature (19.1-20.9°C) and dissolved oxygen (> 7 mg L⁻¹) in 10 kL tanks was measured daily using a Horiba U10 water quality analyser (Horiba, Japan). Total ammonia (TAN < 0.1mg L⁻¹) was measured at the commencement of each experimental run using a rapid colourmetric method (E.Merck, Model 1.08024, Germany). Fluorescent lighting was controlled to provide a 12L:12D photoperiod for the duration of the study.

2.6 Statistical analysis

The effect of diet type and feeding time on plasma glucose concentration was analysed using two-way *ANOVA*. The raw data on plasma glucose concentration was log transformed prior to statistical analysis to correct heterogeneous variances (Cochran's Test). The effect of diet type and feeding time on percentage feed intake (% of body weight) was also analysed using two-way *ANOVA*. Where necessary, significant differences between treatment means were identified using the Student Newman-Kuels multiple comparison procedure. All data was statistically analysed using Statgraphics Plus V4 (Manugistics, Rockville, USA). Alpha was set at 0.05.

3. **RESULTS**

No fish died during the pilot trial and all fish recovered after being returned to holding facilities. Baseline plasma glucose concentrations of unfed yellowtail kingfish were determined to be 3.62mM (i.e. 0h sample; Table 2). Two-way *ANOVA* indicated that log glucose concentration was significantly affected by both factors and their interaction (P<0.0001). The strong interaction was driven by the disproportionate response of fish fed the 40% glucose diet compared to the other test feeds (Figure 1). Therefore, each of the dietary treatments was considered in isolation and one-way *ANOVA* was used to identify when circulating levels of plasma glucose returned to basal levels. Raw and log plasma glucose peaked at 28.3 mM approximately 9-12 hours after consuming the diet containing 40% glucose and returned to basal levels after 24 hours (Figure 1 & 2; Table 2; one-way *ANOVA*, *SNK*). There was no statistical difference (P>0.05) in the log glucose concentration of fish fed the pregelatinised or control diet sampled at different times (Table 2).

In order to more thoroughly investigate changes in the plasma glucose response of fish fed the pregelatinised wheat starch or control diet, the glucose treatment was dropped from the original two-way *ANOVA*. The reduced two-way *ANOVA* indicated that log plasma glucose concentration was significantly affected by time of sampling ($F_{10,18}=3.07$, *P*=0.0185), but not by diet type ($F_{1,18}=2.48$, *P*=0.1325) nor the interaction of diet type and sampling time $F_{10,18}=0.25$, *P*=0.9846). SNK multiple comparisons procedure indicated that there was only 3 significant differences among the pooled log glucose concentrations of fish sampled over time: 3h vs 48h; 9h vs 48h; 18h vs 48h. The data was somewhat inconclusive, but a graphical interpretation of the pooled data (n=4) appears to indicate that there was an elevation in log glucose concentration occurring after feeding, regardless of diet type (Figure 3). The pattern of response was similar in fish fed the pre-gelatinised wheat starch or control diet, but the circulating plasma glucose concentration of fish fed the 40% wheat starch diet was slightly higher than fish fed the control diet (Figure 2 and Figure 3).

During the feeding process, approximately 93% of fish willingly ate their allocated test diet. Consumption of a single meal by individual fish remained relatively constant and ranged from 2.0 to 5.0% of body weight (Table 2). Two-way *ANOVA* indicated that relative feed intake was significantly affected by dietary treatment ($F_{2,26}$ =4.03; P=0.0298), but not by sampling time ($F_{9,26}$ =0.76; *P*=0.6488) nor the interaction of terms ($F_{18,26}$ =0.97, *P*=0.5140). The relative intake of fish fed on the pre-

gelatinised starch diet was significantly higher than that of the other two diets, which were statistically similar (i.e. procedural control = glucose < pre-gelatinised starch; n=18).

4. **DISCUSSION**

The results from this study have demonstrated that a diet containing a highly available source of CHO such as D-glucose causes a significant and relatively rapid elevation in the circulating plasma glucose concentration of yellowtail kingfish. Intake of this diet also caused prolonged hyperglycaemia, with kingfish unable to regulate their circulating plasma concentrations back to basal levels for nearly 24 hours (Figure 1). The generalised response of kingfish to an oral dose of glucose was similar to that recorded in many fish injected with a bolus of glucose, although the uptake of glucose from the digestive tract appears to occur at a slower rate than for direct injection. The rapid uptake and prolonged clearance time in fish fed the glucose diet is most likely due to its highly refined state and the fact that many of the physical and enzymatic processes normally necessary to digest and absorb complex CHO's were not required or bypassed.

In dramatic contrast, the circulating plasma concentration of fish was little affected by the diet containing 40% pre-gelatinised wheat starch. The results also indicated that the time-course response to the 40% wheat starch diet was similar to that of the low CHO control diet. However, although the response to the 40% wheat starch diet was similar, the circulating levels of glucose were slightly higher in this group which probably reflects the increased CHO content of this diet. Minor elevations and fluctuations in the circulating glucose concentrations of fish fed the basal diet likely reflect post-prandial metabolic responses to feeding, digestion and absorption of the diet. Daily rhythms in circulating blood glucose levels have been studied in several species of fish and have also been found to correlate highly with environmental influences such as photoperiod or feeding schedules (Cerda-Reverter et al. 1998; Pavlidis et al. 1999; Lopez-Olmeda et al. 2009). While these variables were not investigated in this trial, the similarity in the way these two response curves fluctuated would appear to indicate that the digestive process may be responsible for the relatively slight rise and fall in circulating glucose levels rather than environmental cues.

The ability of fish to efficiently digest CHO relies heavily on the molecular complexity and amount of processing (Wilson 1994; Lee et al. 2003). Although pre-gelatinised wheat starch is comprised almost exclusively of linked glucose-monomers, the diet containing an extreme level of this ingredient had little effect on the circulating glucose concentration in kingfish. When calculated on a relative weight basis and assuming the digestibility of glucose is 90% (Furuichi, Taira and Yone 1986), the satiated dose rate of fish fed the 40% glucose diet was approximately 11.5 g D-glucose kgBW⁻¹. Similarly, if the digestibility of glucose from wheat starch is assumed to be approximately 50% (Furuichi et al. 1986), fish fed the wheat starch diet received an effective dose rate of approximately 2.0g D-glucose kgBW⁻¹. This indicates that fish receiving the glucose diet may have been flooded with approximately 5.8 times the available glucose than received by fish offered the wheat starch diet. If realistic, the dramatic reduction in the digestibility and therefore the dose rate of available glucose could explain the major difference in the peak concentration and duration of these two response curves (Figure 1). The determination of apparent digestibility coefficients for yellowtail kingfish fed CHO sources such as wheat will be necessary to accurately interpret data such as that generated from this and future feeding studies. In addition, the peak response of fish fed the glucose treatment was close to 30 mM which is close to the stated upper range of the glucose meter. Therefore, further studies employing this instrument will need to ensure that oral doses or intra-peritoneal and intra-muscular injections of glucose do not exceed 10 to 11 g D-glucose kgBW⁻¹.

Using individual fish as replicates in feeding experiments is often desirable from a statistical point of view but can be problematic from a practical point of view. Many fish exhibit schooling behaviours which are necessary to promote acceptable feed intake and reduce stress, particularly in gregarious species such as mulloway *Argyrosomus japonicus*, where stocking density has been shown to influence performance characteristics (Pirozzi et al. 2008). In this experiment, individually housed yellowtail kingfish starved for a period of 48h consumed an average of 2.89, 3.18 or 3.87% body

weight of the control, glucose or pre-gelatinised diets, respectively (n=18). This encouraging result indicates that it is possible to individually house and feed fasted yellowtail kingfish for use in experiments. It would be advantageous to compare the basal glucose levels and feed intake of yellowtail kingfish housed individually with those of fish in larger schools to evaluate the effects of stocking density on these response variables.

5. CONCLUSIONS

- Prior starved yellowtail kingfish fed a diet containing 40% D-glucose to apparent satiation once daily exhibited a rapid increase in plasma glucose concentration and a prolonged state of hyperglycaemia which lasted for approximately 24 hours.
- Although slightly elevated, the glycaemic response of yellowtail kingfish fed a diet containing 40% by weight of pre-gelatinised wheat starch once daily to apparent satiation was not dissimilar to fish fed a high protein, low carbohydrate procedural control
- Starved, individual housed yellowtail kingfish readily accepted experimental diets following acclimation in 200L experimental cages

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TABLE 1

Ingredient (g kg ⁻¹)	40% pre-gelatinised wheat starch	40% D-glucose	Procedural control
Peruvian fishmeal	237	237	397
Meat meal	90	90	150
Poultry meal	60	60	100
Fish oil	30	30	50
Dehulled lupin	120	120	200
Extruded wheat	60	60	-
Vitamin/mineral premix	3	3	3
Pre-gelatinised wheat starch*	400		
D-glucose**		400	
High protein corn gluten*			100

Ingredient composition of test diets (g kg⁻¹ dry basis)

All protein sources, fish oil and premix supplied by Ridley Aquafeed Pty Ltd, Narangba, QLD.

 * Pre-gelatinised wheat starch and corn gluten provided by Penford Australia Ltd., Lane Cove NSW, Australia
 ** D-glucose 99.5% (SIGMA-Aldrich Pty Ltd, Castle Hill NSW, Australia) provided by University of Technology, Sydney (UTS)

TABLE 2

	40% wheat starch		40% glucose		Control	
	mM	%BW	mM	%BW	mM	%BW
0	3.80	-	3.21 ^a	-	3.85	-
1	5.00	3.70	6.20 ^b	2.68	5.15	3.25
2	4.75	2.89	12.95 ^c	3.28	5.35	2.75
3	7.70	5.38	13.85 ^c	2.42	6.75	2.38
6	5.90	4.35	16.90 ^c	3.11	4.75	2.00
9	7.80	3.75	28.30^{d}	3.27	5.40	2.32
12	6.30	2.61	25.95 ^d	2.94	4.60	4.62
18	6.85	3.67	18.45 ^c	2.53	6.15	2.83
24	5.15	4.37	3.30 ^a	4.20	3.95	2.84
36	4.40	2.83	3.60 ^a	3.61	3.35	2.58
48	3.15	5.13	3.25 ^a	3.73	2.85	3.36
Pooled sem	1.58	1.10	1.64	0.56	1.46	0.31

Plasma glucose concentration (mM) and percentage feed intake (%BW) of juvenile yellowtail kingfish fed experimental diets once to apparent satiation (n=2)

Data values are actual data. *ANOVA* was performed on log transformed data to correct heterogeneous variances. Different letters in superscript indicate significant differences between row means for each diet treatment.



- Glucose
- Pregel wheat starch
- Control

FIGURE 1

Raw plasma glucose concentration (mean \pm sem; n=2) in yellowtail kingfish fed diets containing 40% glucose (closed squares), 40% pre-gelatinised wheat starch (open circles) or a low carbohydrate control diet (closed triangles).



- Glucose
- Pregel wheat starch
- Control

FIGURE 2

Log transformed plasma glucose concentration (mean \pm sem; n=2) in yellowtail kingfish fed diets containing 40% glucose (closed squares), 40% pre-gelatinised wheat starch (open circles) or a low carbohydrate control diet (closed triangles).



FIGURE 3

Pooled log transformed plasma glucose concentration (n=4) in yellowtail kingfish fed diets containing 40% pre-gelatinised wheat starch or a low carbohydrate control diet.

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9.6 Abstract presented by Michael Moses at Asian Pacific Aquaculture 2009, 3-6 November 2009, Kuala Lumpur, Malaysia

CARBOHYDRATE UTILISATION IN YELLOWTAIL KINGFISH Seriola lalandi

Michael Moses^{*}, Mark A. Booth, Geoff L. Allan and David Booth

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Yellowtail kingfish (YTK), *Seriola* lalandi, is an active pelagic carnivore with a high metabolic rate. The species is currently farmed in southern Australia and members of the same genus are farmed extensively in Japan and other Asian countries. YTK are usually fed high protein extruded pellets (e.g. 40-50% crude protein, 15-20% crude lipid) and depending on the consumer market, often fed diets devoid of land animal protein sources. In many cases this has meant diets are manufactured almost exclusively from fish meal and fish oil. Because these resources are under constant and increasing pressure from a diverse range of animal and human nutrition sectors there is renewed interest in the ability of *Seriola* spp. to utilise alternative protein and energy sources, particularly carbohydrates (CHO). We postulated that if YTK is able to effectively utilise CHO it may allow a reduction in dietary protein content or other energy sources and therefore the flexibility and cost of diet formulations.

In this study, results from two experiments are reported. In the first, the uptake and clearance rate of glucose from the blood stream was measured following an intra-peritoneal injection of 1 g D-glucose kg BW⁻¹. In the second experiment, a summit-dilution approach was used in which a high performance reference diet (Ref) of 54% crude protein, 14% crude lipid (21 MJ kg⁻¹) was progressively replaced with 10, 20, 30 or 40% of either extruded wheat (Ext. wheat), gelatinised wheat starch (Starch) or an inert filler, diatomaceous earth (D. Earth). Diets were fed restrictively based on body weight to ensure fish could not consume more feed to compensate for dietary deficiencies. Our results demonstrated that in contrast to expectations, YTK are able to rapidly absorb and clear glucose from their bloodstream (Fig A). Increased growth of YTK fed an equivalent content of extruded wheat or gelatinised wheat starch compared with diatomaceous earth indicates utilisation of these ingredients (Fig B). In this respect, they behave more like omnivorous species than carnivorous species. This helps explain why YTK performed well on diets containing up to 30% extruded wheat or pre-gelatinised wheat starch in the diet. These data indicate YTK are able to utilise CHO for energy and suggest that reduction of protein content may be possible without reducing performance.



9.7 Abstract presented by Igor Pirozzi at the XIII International Symposium on Fish Nutrition and Feeding 1-5 June, 2008, Florianopolis, Brazil

Session II - Nutrient Requirement and Availability



DEFINING THE DIETARY PROTEIN AND ENERGY NEEDS OF MULLOWAY: INVESTIGATIONS USING FACTORIAL BIOENERGETIC APPROACHES TO NUTRIENT REQUIREMENTS

Pirozzi, 1.1; Booth, M.A.2; Allan, G.L.2

¹James Cook University (JCU), Australia; ²NSW Department of Primary Industries Port Stephens Fisheries Centre, Australia

Mulloway (Argyrosomus japonicus) are a commercially and recreationally important sciaenid species in Australia. Aquaculture production of mulloway continues to increase with fish grown predominantly in sea-cage operations in the states of New South Wales (NSW) and South Australia (SA). As a relatively new aquaculture species, little is known of mulloway's nutritional requirements and performance under various culture regimes. Research efforts have focused on improving fingerling production, evaluating the potential of different feed ingredients, deriving growth models and identifying nutrient and energy requirements. Bioenergetic models have become more prevalent in fish nutrition over recent years. These models are usually derived from a series of empirical experiments and good data on growth potential. However, several assumptions are often made with these models, especially in their simplest forms, that can influence the predictions they make. For example, the effect of temperature on metabolic weight exponents is often ignored as too is its potential effect on nutrient utilization. Utilization coefficients are often derived from studies with relatively small size class of fish and are often expressed as constant values in bioenergetic models. This may not be appropriate when making predictions on the requirements of larger fish. In addition, bloenergetic models are mostly derived from data based on feed intake and, if feed intake is suboptimal for any reason, predictions will be spurious. This study details research aimed at determining the dietary digestible protein and energy requirements of mulloway. Several studies were conducted to establish parameters describing maintenance demands for protein and energy, protein and energy utilization efficiencies, body composition and growth. These parameters comprise the main components driving the bioenergetics models. Data were collected over a wide range of temperatures and fish sizes and using commercial and experimentally prepared feeds. The routine metabolic rate of mulloway was also established through oxygen consumption trials over a range of temperatures and fish sizes to complement the energetic profiles established through analyses of carcass composition. Factorial modelling of these data gave estimates of the dietary protein and energy requirements for mulloway and also allowed estimations of optimal digestible protein (DP) to digestible energy (DE) ratios throughout the production range. Piecewise linear analysis identified key growth stages which in turn allow the theoretical assignment of diets based on the predicted DP:DE requirements. While factorial modeling can be a useful tool in estimating nutritional requirements it is important however to be aware of the inherent limitations. The robustness of any model is clearly dependent on its sensitivity to the underlying assumptions of the derived data sets; the implications of these assumptions are discussed.

KEYWORDS: mulloway, Argyrosomus japonicus, growth, bioenergetics, protein, energy requirements.

XIII ISENE - International Symposium on Fish Nutrition and Feeding - June. 2008 - FLORIANÓPOLIS, BRAZIL

DRAFT

Research Fact Sheet

AQUAFIN CRC: FEED TECHNOLOGY FOR TEMPERATE FISH SPECIES

Research Partners: Ridley Aqua-Feed Aquafin CRC FRDC NSW DPI Clearwater Mulloway

> Species under Research:

igb Performance Aquatic Nutrition











Australian Government Fisheries Research and



al 1007 NSW DEPARTMENT OF PRIMARY INDUSTRIES



Why do this research?

This research follows on from the very successful project "Increasing the profitability of snapper farming by improving hatchery practices and diets". The research in this project was listed as a priority area for research by the Aquafin CRC Joint Management Advisory Committee, The Yellowtail Kingfish Aquaculture Strategic R&D Plan 2003-2008 and at the Aquafin CRC Snapper Workshop, 2003. It has been requested by marine fish farmers in NSW and SA and by the largest feed manufacturer in Australia – Ridley Agriproducts. Two PhD students from James Cook University were appointed to work on this project at Port Stephens Fisheries Centre (PSFC) and their research will be profiled in the next Fact Sheet.

Over \$3 million will be injected into this project over 4 years: \$977.905 from FRDC

\$380,567 from industry partners \$1,851,489 from NSW .

DPI

Facilities & staff at PSFC:

Research facilities at PSFC are second to none and include spawning and larval rearing tanks, production units for algae, rotifers and brine shrimp and lined ponds for extensive larval rearing. Dr Geoff Allan is Principal Investigator on the project assisted by Research Scientists Drs Stewart Fielder and Mark Booth. The team of 7 technicians will be

Introducing... The scientists from NSW DPI



Introducing... Our industry partners



introduced to you in subsequent Fact Sheets.

Objectives:

3

- 1. To reduce costs of fingerling production. To improve the costeffectiveness of grow
 - out diets. To validate improved feeds and feeding practices on a commercial scale.

Experiments:

About 30 experiments are scheduled for completion by May 2009. These will be conducted at NSW DPI's Port Stephens Fisheries Centre, Taylors Beach. Future Fact Sheets: Research Fact Sheets in this series will provide a brief description of completed experiments and results.

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Aquafin CRC Feed Technology Final Report, Vol. 2, Booth et al.



Research Fact Sheet

FACT SHEET Nº 2.

Hieb Performance Assatic Nutrition

AQUAFIN CRC: FEED TECHNOLOGY FOR TEMPERATE FISH SPECIES

Research Partners:

Ridley Aqua-Feed Aquafin CRC FRDC NSW DPI Clearwater Mulloway









Research profiles of James Cook University PhD students

The title of Debra Ballagh's project is "Improvement of intensive rearing of larval and juvenile mulloway (Argyrozomus japonicus) and yellowtail kingfish (Seriola lalandi lalandi)." The major aims of her research are:

 To determine the best combination of live feeds/inert feeds for larval mulloway and yellowtail kingfish.
 To determine the best wearing and feeding

strategies for juvenile mulloway and yellowtail kingfish.

Why do this research?

A reliable supply of cheap, high quality healthy fingerlings is essential for sustainable mulloway and yellowtail kingfish farming. Fingerling costs for mulloway and kingfish are currently estimated at \$0.60-\$2.00/fingerling. This cost may represent approximately 10% or more of the total production cost. To improve profitability, there is a need to reduce the cost and improve the quality and vigour (optimize growth) of fingerlings and to develop cost-effective high-performance diets and feeding systems for both hatchery and grow-out. This project will improve hatchery methods and potentially replace live





PhD Students Debra Ballagh and Igor Pirozzi

feeds, including artemia which are expensive and can have unreliable supply, with alternative live feeds or artificial feeds. The project will also determine feeding strategies (frequency of feeding, density of fish, artificial day-length) to optimise fingerling survival, growth, quality and population size variation. Cost of production will be reduced by increasing the turnover rate of fingerlings from the hatchery-nursery systems and thus allow more hatchery runs to be done each year.

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The title of Igor Pirozzi's project is "Comparison of protein and energy requirements of mulloway and kingfish using a factorial modeling approach". The major aims of his research are:

 To construct and test factorial models based on the protein and energy requirements of rapidly growth mulloway and kingfish.
 To compare differences in requirements for

protein and energy for yellowtail kingfish and mulloway.



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Why do this research?

Lack of nutritional information on mulloway and kingfish in Australia is limiting the development of their aquaculture potential. At the moment farmers rely on commercial diets formulated for other species. These diets produce results, but it is unknown if they are nutritionally adequate, especially for rapidly growing fish. Generally, the most important initial nutritional information for diet development is the determination of specific digestibility coefficients for potential feedstuffs. Secondly, the gross nutrient requirements for a fish species needs to be established. Because of the implications for the cost of diets, the requirements for protein and energy are usually determined before requirements for other nutrients. In addition, farmers demand feeds that are highly efficient in terms of feed utilization and that produce lower nutrient outputs from their

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farms.

Debra and Igor are conducting their research at Port Stephens Pisheries Centre. They are on track to finish their PhD's this year and results of individual experiments will be presented in subsequent Research Fact Sheets.





RIDLEY AQUA - FEED High Refermance Agnatic Nucrition.

Research Fact Sheet

FACT SHEET N° 3

AQUAFIN CRC: FEED TECHNOLOGY FOR TEMPERATE FISH SPECIES

EFFECTS OF DIFFERENT PHOTOPERIODS AND FEEDING FREQUENCIES ON GROWTH RATE OF JUVENILE MULLOWAY

Introduction

Determination of the optimal conditions for juvenile fish is necessary to maximize production and efficiency in hatcheries. The combined effects of photoperiod and feeding frequency have been studied before with optimal combinations resulting in significantly improved growth rates. While determining the optimal condition for growth, parameters such as body composition, feed conversion ratios and fish condition also need to be examined to ensure the most efficient method of producing high growth rates is determined.

Results and Discussion

Overall it was found that the photoperiod 18:6 provided the best growth as fish had a significantly high condition factor to fish that had similar weight in the 12:12 photoperiod. The 24:0 photoperiod led to significantly lower weights and poorer feed conversion ratios than other photoperiods and significantly shorter fish. This suggests that 24 hours of light is not suitable or efficient for producing high quality mulloway fingerlings. The reduced growth rate of fish held in 24 h of light may have been due to stress and an elevation in cortisol levels. Cortisol can lead to a reduction in growth rates as it is often positively correlated with hyperglycemia which occurs when cortisol provides free amino acids via inhibition of protein synthesis or active protein catabolism, which can be used in glyconeogenesis. The amino acids being utilized to induce hyperglycemia are no longer used for growth. Subsequently, it is recommended that future studies include measurements of blood cortisol and alucose.

It was also found that there were no significant differences between the 1 h, 3 h, 6 h and 12 h feeding regimes and no significant differences between the FCR of any of the feeding frequencies,



Australian Governmen Fisheries Research and Development Corporation NSW DEPARTMENT OF



which provides useful information for production protocols and will mean a less labour-itnesive approach to rearing juvenile mulloway. It is recommended that fish be fed more than once a day, as those fed once every 24 h were lighter than other feeding regimes. FCRs were similar between the different photoperiods indicating that this difference occurred due to less food consumption.

The fish exposed to a 12:12 photoperiod and 12 h feeding frequency were fed once at 0800 hrs (light) and again at 2000 h (dark). The weight of these fish compared to the weight of fish fed only at 0800 hrs (12:12, 24 h) was significantly higher. This implies that fish were feeding in the dark.

As the fish held in the 12:12 photoperiod were significantly longer than fish in other photoperiods, future light intensity experiments may also be used to examine length differences when fish are held in photoperiods with long dark regimes. Tables summarizing these results are on the next page.



Fisheries Technician Luke Vandenberg showing off one of the prized mulloway broodstock at Port Stephens Fisheries Centre, Taylors Beach, NSW.

Results.

Photoperiod = hours of light Feed regime = hours between feeds

Final weight, length, feed conversion ratio (FCR), condition factor (CF), specific growth rate (SGR) and coefficient of variation for weight (CVwt) (means ± SEM, n=4) of mulloway from individual treatments after 30 days

Photo- period	Feed regime	Weight (g)	Length (cm)	FCR	CF	CVwt
12	1	7.82 ± 0.22	8.9±0.1	1.08 ± 0.05	33.20 ± 0.21	0.18 ± 0.01
	3	7.81 ± 0.17	8.9 ± 0.1	1.16 ± 0.10	33.58 ± 0.99	0.18 ± 0.02
	6	7.44 ± 0.26	8.7 ± 0.2	1.13 ± 0.06	33.05 ± 0.65	0.19 ± 0.01
	12	7.78 ± 0.40	8.8±0.2	1.16 ± 0.08	33.53 ± 0.55	0.17 ± 0.01
	24	6.31 ± 0.18	8.1 ± 0.1	1.34 ± 0.08	33.47 ± 0.33	0.18 ± 0.01
18	1	7.38 ± 0.47	8.3±0.2	1.17 ± 0.03	36.56 ± 0.81	0.17 ± 0.01
	3	8.32 ± 0.30	8.7±0.2	1.25 ± 0.04	36.98 ± 0.38	0.18 ± 0.01
	6	8.12 ± 0.27	8.6 ± 0.0	1.20 ± 0.01	37.39 ± 0.92	0.17 ± 0.02
	12	7.43 ± 0.35	8.3 ± 0.2	1.31 ± 0.06	36.90 ± 0.27	0.17 ± 0.02
	24	6.48 ± 0.14	7.9 ± 0.1	1.21 ± 0.02	36.05 ± 0.19	0.16 ± 0.01
24	1	6.95 ± 0.25	8.1 ± 0.1	1.42 ± 0.02	37.21 ± 1.05	0.15 ± 0.01
	3	6.69 ± 0.16	7.9 ± 0.1	1.47 ± 0.06	37.53 ± 0.33	0.18 ± 0.00
	6	7.38 ± 0.10	B.1 ± 0.1	1.61 ± 0.08	38.52 ± 0.49	0.17 ± 0.01
	12	6.67 ± 0.14	8.1±0.1	1.44 ± 0.08	35.24 ± 0.30	0.16 ± 0.01
	24	5.39 ± 0.09	7.4 ± 0.1	1.42 ± 0.03	35.41 ± 1.54	0.15 ± 0.01

Mean weight, length, feed conversion ratio (FCR), condition factor (CF), specific growth rate (SGR) and coefficient of variation for weight (CVwt) of multioway from varying photoperiods and feed regimes

Main offect	Level	Weight (g)	Length (cm)	FCR	CF	CVwt
Photoperiod	12	7.43 ± 0.29^9	8.7 ± 0.2"	1.18 ± 0.07 *	33.37 ± 0.55	0.16 ± 0.01
	18	$7.54\pm0.32^{\text{s}}$	8.4 ± 0.1^9	$1.23\pm0.03^{\ \text{d}}$	36.78 ± 0.52	0.17 ± 0.01
	24	6.61 ± 0.33^9	$7.9\pm0.1^{\rm c}$	1.47 ± 0.08^{u}	$\frac{36.78}{10} \pm 0.74$	0.18 ± 0.01
Feed regime	1 3 6 12 24	7.38 ± 0.25^{8} 7.60 ± 0.46^{8} 7.85 ± 0.24^{8} 7.29 ± 0.32^{8} 6.05 ± 0.34^{7}	8.4 ± 0.3^{4} 8.5 ± 0.3^{4} 8.5 ± 0.2^{8} 8.4 ± 0.2^{8} 7.8 ± 0.3^{7}	1.22 ± 0.04 1.29 ± 0.07 1.31 ± 0.05 1.31 ± 0.07 1.32 ± 0.04	35.66 ± 0.69 36.03 ± 0.57 36.32 ± 0.68 35.22 ± 0.38 34.98 ± 0.89	$\begin{array}{c} 0.17 \pm 0.01 \\ 0.18 \pm 0.01 \\ 0.18 \pm 0.01 \\ 0.17 \pm 0.02 \\ 0.17 \pm 0.01 \end{array}$

¹Within such factor and column, values (level means ± SEM; n=20 for photoperiod, n = 12 for feed regime) with the same letter in the superscript are not significantly different (P>0.05; two-way ANOVA, SNK) ² There were no significant interactions (P>0.05) between photoperiod and feed regime for any of the growth

indices