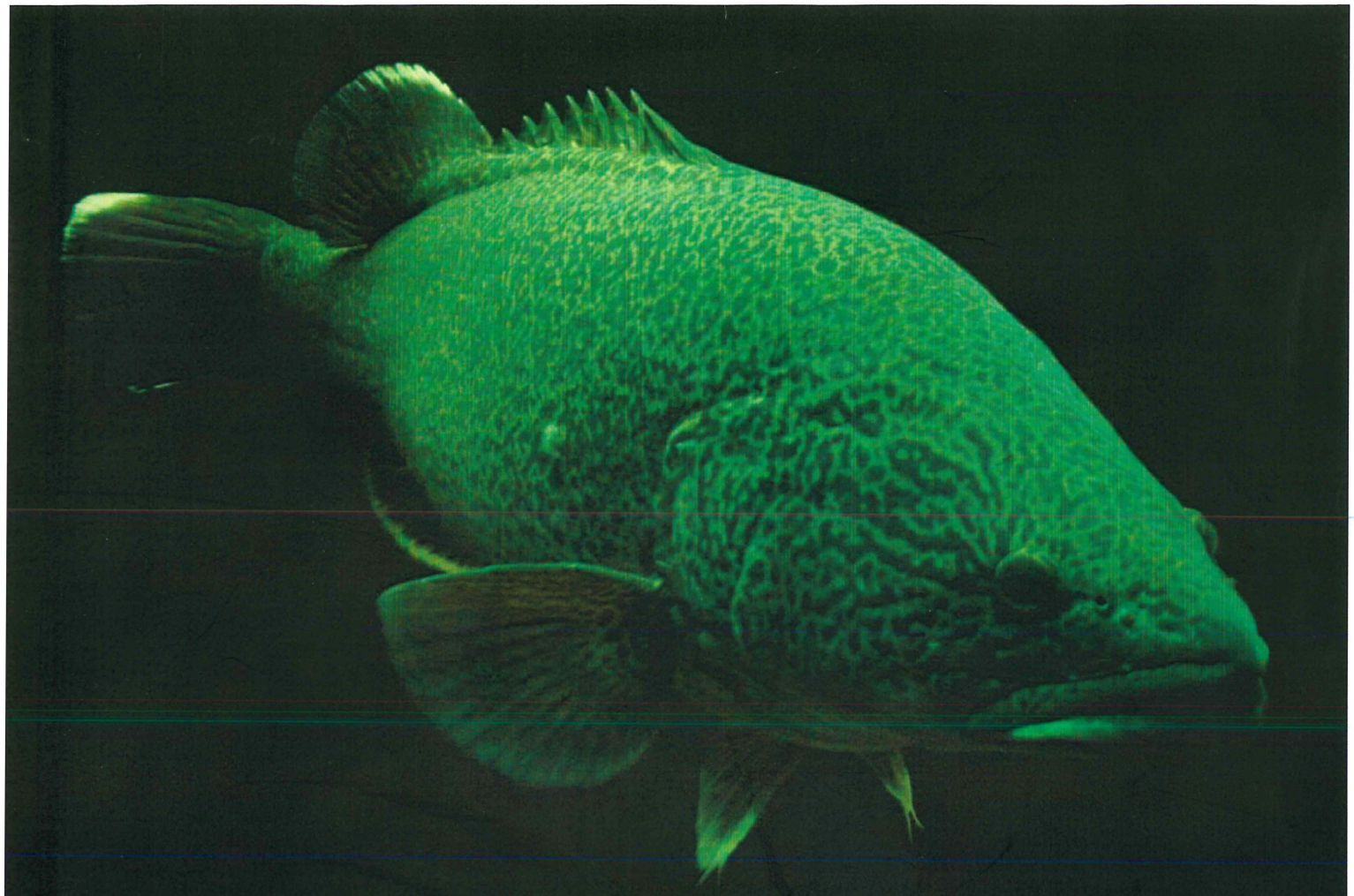

DEVELOPMENT OF INTENSIVE COMMERCIAL AQUACULTURE PRODUCTION TECHNOLOGY FOR MURRAY COD

Edited by

B.A. Ingram and S.S. De Silva

State Government Victoria Department of Primary Industries

Australian Government
Fisheries Research and Development Corporation

DEAKIN UNIVERSITY

Project No. 1999/328

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B.A. Ingram[1] and S.S. De Silva[2] (Editors)

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June 2004

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Published by Primary Industries Research Victoria, Marine and Freshwater Systems, Department of Primary Industries, Snobs Creek, Victoria, 3225.

ISBN 1 74146 275 4

Formatted/designed by Primary Industries Research Victoria Queenscliff

Printed by PIRVic Snobs Creek, Victoria

Cover photograph by James Bailey

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NON-TECHNICAL SUMMARY

1999/328 Development of Intensive Commercial Aquaculture Production Technology for Murray Cod

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Objectives:

1. To develop and evaluate Best Practice husbandry, nutrition and fish health for commercial production of Murray cod under extensive pond-based hatchery, and intensive tank-based growout conditions.

2. To develop and implement an appropriate extension and associated market strategy to ensure effective and efficient transfer of research outcomes and associated protocols and technologies to industry.

Non Technical Summary:

Murray cod, *Maccullochella peelii peelii* (Mitchell) is highly valued for recreational, commercial and conservation purposes. However, due to concerns of declining populations in the wild, commercial fishing for Murray cod is now banned and regulations limit the take by recreational anglers. State government and private fish hatcheries currently produce up to 1.8 million fingerlings annually for stock enhancement programs and grow-out purposes. In recent years, there has been considerable interest in the grow-out of Murray cod to satisfy market demands. However, information on the aquaculture of Murray cod is lacking. The present study was developed to address this and to facilitate industry development.

Murray cod aquaculture is a small but growing industry in Australia. Most production occurs in recirculating aquaculture systems (RAS). On-farm production has ranged from < 1 t/annum/farm to over 100 t/annum/farm. The level of production of market-sized Murray cod has increased substantially and over 100 tonne was produced in 2000/01. The total value of the Murray cod industry (hatchery production of fingerlings and table fish production combined) was $2.48 million/annum in 2001/2002, with the grow-out sector representing 80% of the total industry value.

The present study has considerably broadened the knowledge on the culture of Murray cod particularly the weaning of post-larvae and fingerlings, and grow-out in tanks and cages under controlled and ambient conditions. This species performs well especially in RAS, as indicated by excellent growth rates, survival rates and food conversion rates (FCR's) at high stocking densities. Key production information for the grow-out of Murray cod to a minimum market-size (700 g) is described. Based on these data, in a commercial operation, for every 1,000 fingerlings (1g/fish) stocked into a RAS, operating at a temperature between 22-26°C, between 413 and 616 kg (median 500 kg) of Murray cod (at 700 g/fish) are expected to be harvested

from 36 weeks to 108 weeks after stocking. Information collected during cage culture trials showed that Murray cod, at least stocked as "young-of-year", can be reared in cages under ambient conditions.

Murray cod requires a dietary protein content of about 50% for optimal growth performance, and is able to effectively digest food industry by-products, such as defatted soybean meal, shark meat meal and meat meal. The "protein sparing" abilities of Murray cod are limited, but up to 32% of the fish meal content in Murray cod diets could be replaced without compromising growth performance. The present study has provided information to develop a cost- effective commercial diet for intensive Murray cod culture, which was successfully tested under commercial conditions. Adoption of this diet for grow-out of Murray cod in these systems will significantly reduce the overall cost of production.

Values for 16 water quality parameters are summarised to provide a guide to acceptable ranges for commercial intensive Murray cod aquaculture. A simplified nutrient mass balance model was developed to estimate the amounts of nitrogen (N) and phosphorus (P) discharged from an intensive Murray cod aquaculture system. Based on median nutrient input values, for every tonne of market size Murray cod harvested approximately 12.4 kg P/tonne and 67 kg N/tonne are produced. Changes in FCR and the amount of N and P in the feed strongly influenced these values. Results from the present study also have implications for the future construction, operation and management of RAS systems for the culture of Murray cod.

The known fish diseases and fish health issues affecting Murray cod are described. Strategies to assist farmers in the management of the health and well being of stock in intensive aquaculture facilities are discussed. These include water supply sources and treatment, quarantine and management of new stock, management of hygiene in aquaculture facilities, monitoring stock and identifying health problems and use of drugs and chemicals. A decision support pathway is presented to assist farmers in the identification and management of major health problems in Murray cod aquaculture operations.

A variety of Murray cod products are now routinely cultured for use in a range of markets from post-larvae and fingerlings for stock enhancement and grow-out, to table fish (0.6 – 4.0 kg) sold live, fresh, gilled and gutted, and as fillets into a number of markets. Fingerlings have been exported for grow-out purposes, and Murray cod products have been sold in international markets. There has been improved market chain efficiency through more reliable and routine supply of products of known quality. Prices paid for farmed Murray cod over the past three years have declined from a high of approximately $24.00/kg in late 1999 to a steady price of around $13.50/kg. Information is provided on the marketing of Murray cod from an industry standpoint. An industry driven Murray cod marketing co-operative is proposed.

The financial and economic efficiencies are investigated for five Murray cod RAS (1, 5, 25, 50 or 100 tonne/annum). Production data from industry are combined with results from the present study and analysed using AQUAFARMER™ feasibility software to determine and evaluate the bio-economic performance of each system. The highest running costs were labour (27%), feed (26%), seedstock (16%). Each of the farms revealed strong indicators of the inherent financial viability of growing Murray cod in RAS, though configuration of annual running costs and sale price are strongly affected both internal rate of return (IRR) and profit margin (PM). The study showed that on-farm diversification would improve viability of smaller farms while there were significant opportunities for improved risk management in larger systems and that economies of scale can be achieved. This study indicated that options to improve profitability of production included adoption of more energy and labour efficient RAS technology, improved husbandry to optimise growth and survival, use more cost effective diets and use of genetically improved seedstock with enhanced growth, survival and FCR's.

Future R&D needs for the industry are discussed. These include:

- *Production systems*: Improve efficiency and reliability of RAS. Develop and assess cage and pond culture techniques in association with IAAS.
- *Broodfish and seedstock*: Develop guidelines for procurement of wild fish and management of broodfish and breeding programs for production of seedstock used in stock enhancement programs. Develop management guidelines for closed breeding systems in which self-propagated broodfish are used for production of seedstock for the grow-out industry, taking into account genetic requirements of selected breeding programs. Increase production and availability of seedstock. Develop and refine controlled spawning techniques, including 'out-of season" spawning.

- *Genetics and biotechnology*: Establish a selective breeding program to ensure the long term genetic integrity and improvement of farmed Murray cod stocks. Apply chromosome manipulation, sex manipulation and hybridisation techniques to Murray cod aquaculture and assess performance of progeny and associated value to selective breeding programs. Establish a cryopreserved sperm bank for use in selective breeding and biodiversity conservation programs.
- *Nutrition:* Explore options for reducing both feed costs and dependency on fish meal and fish oil, through use of alternative protein and oil rich ingredients. Develop feed management strategies for intensive aquaculture systems. Develop environmentally cleaner diets that have reduced P and N wastes.
- *Fish health and water quality*: Develop effective on-farm biosecurity programs and fish health management strategies. Evaluate new, alternative and safe methods of controlling diseases in farmed Murray cod, including the use of probiotics. Determine optimal ranges and critical levels of water quality parameters that affect fish health and growth.
- *Marketing and bio-economics*: Develop appropriate marketing and promotion strategies. Monitor markets and develop value-added products that meet market demands/expectations. Develop codes of practices and HACCP programs that ensure product quality. Maintain, refine and update bio-economic models for Murray cod aquaculture.

A vertically integrated Murray cod aquaculture industry is envisaged for Australia, with some elements, already in place. The industry is expected to expand as new operators enter the industry and production methods and operations are improved. Planning for the growth and marketing of the increasing supply of aquaculture products, including Murray cod, is essential at a national, state and regional level. Industry needs to embrace ESD principles and promote best management practices and quality assurance programs across all phases of production. For the industry to make substantial increases in production and market access, both domestically and internationally, requires corporate investment in large-scale production. Production using RAS is a capital-intensive endeavour and for the most part requires corporate investment. Nevertheless, there is still a place for owner-operator farms, and use of integrated agri-aquaculture systems (IAAS), particularly in rural areas, which are in a position to supply local and/or specialised niche markets.

Murray cod aquaculture is a fledgling industry and will continue to need R&D funding support from government to foster growth in the short to medium term. Government needs to continue on-going support through provision of extension services, reliable and streamlined licensing and permitting processes, clearly defined and consistent regulations and enforcement processes. Government has an important role to play in promoting aquaculture developing markets and attracting corporate investment. R&D must be directed towards solution of practical problems and impediments facing the industry. Government aquaculture managers, research providers and industry need to recognise that not all problems have technical solutions, some problems warrant the efforts of organisations and institutions at the level of policy formation to create a climate that fosters industry development.

Keywords:

Australia, freshwater, *Maccullochella peelii peelii*, aquaculture, industry status, development, nutrition, water quality, diseases, fish health, marketing, economics, intensive, recirculating.

ACKNOWLEDGMENTS

The authors wish to gratefully acknowledge the funding support of the Fisheries Research and Development Corporation and Fisheries Victoria (Victorian Department of Natural Resources and Environment) for this project. Significant in-kind contributions from Deakin University (Victoria) and the Murray cod aquaculture industry are also gratefully acknowledged.

Further acknowledgments for the specific participants and associated supporters of the various components of this project are provided in the respective chapters of this report. The collective contributions to this project of these people and the actual project team of researchers and technicians, the latter of whom have mostly contributed to the preparation of this report as authors of various chapters, are also gratefully acknowledged by the Principal Investigator and his R&D colleagues at Primary Industries Research Victoria and Deakin University, Warrnambool.

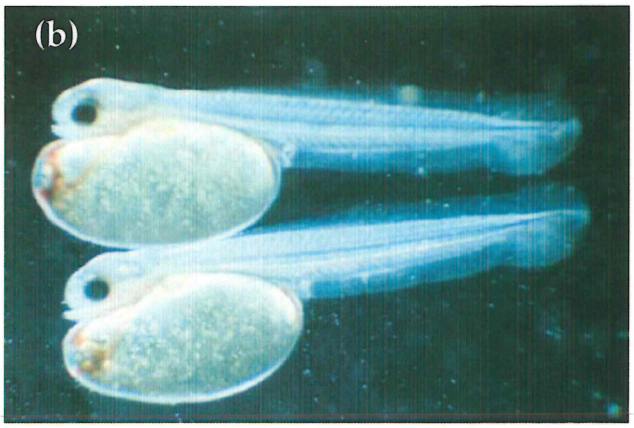

Plate 1

(a) Large, wild Murray cod (Photo: J. Douglas, PIRVic). (b) Newly hatched Murray cod larvae (Tl = 7.8 mm). (c) Harvesting a drained fry pond at PIRVic, Snobs creek. (d) Murray cod fingerlings being collected from the sump of a fry pond. (e) A Murray cod recirculating aquaculture system at Australian Aquaculture Products (AAP), Euroa, Victoria. (f) Hand grading farmed Murray cod at AAP.

Plate 2

(a) Hand feeding Murray cod fingerlings. (b) Weaned, tank-reared Murray cod fingerlings. (c) Murray cod being stocked into a tank for a commercial scale feed trial at AAP. (d) Intensively-reared market size Murray cod. (e) Floating cage (lower right) holding Murray cod at the Torrumbarry Silver Perch farm, Victoria. (f) Hand feeding Murray cod in cages attached to a floating pontoon at PIRVic, Snobs Creek.

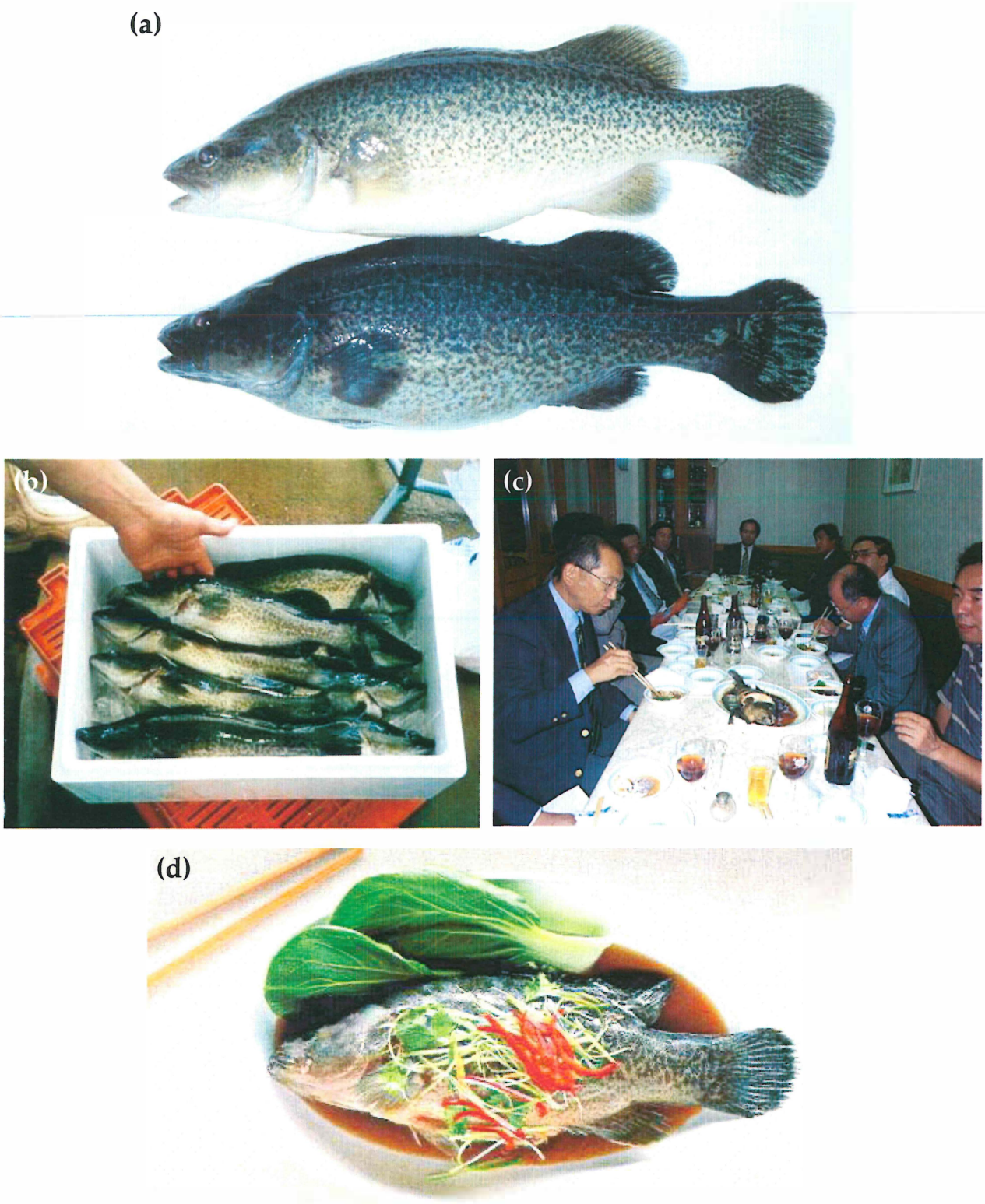

Plate 3

(a) Colouration of Murray cod reared under different conditions: Upper - reared in cages in an earthen pond, lower – reared in an intensive recirculation system (see Chapter 3). (b) Farmed Murray cod ready for market. (c) A banquet featuring Murray cod. (d) Steamed farmed Murray cod ready to serve.

1 MURRAY COD AQUACULTURE

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Ingram, B.A., De Silva, S.S. and Gooley, G.J. (2004). Murray cod aquaculture. In: *Development of Intensive Commercial Aquaculture Production Technology for Murray cod* (ed. B.A. Ingram and S.S. De Silva), pp 1-14. Final Report to the Fisheries Research and Development Corporation (Project No. 1999/328). Primary Industries Research Victoria, DPI, Alexandra, Victoria, Australia.

CONTENTS

1.1 Introduction

The Murray cod, *Maccullochella peelii peelii* (Mitchell), is a member of the family Percichthyidae, which contains some 30 species found in marine, brackish and freshwaters in tropical and temperate regions of the world (www.fishbase.org). In Australian freshwaters the family is represented by seven species and one subspecies in two genera; the cods (*Maccullochella* spp.), and the basses and perch (*Macquaria* spp.) (Harris and Rowland 1996). Murray cod are grouper-like in appearance with a dark, mottled olive-green to dark green body pattern. The head has a concave profile and the mouth is relatively large as is typical of predatory fish (Plate 1a). Their diet includes molluscs, crustaceans, fish and other aquatic and semi-aquatic vertebrates (Harris and Rowland 1996). Murray cod are highly valued for recreational, commercial and conservation purposes. It is sought after as a table fish and up until recently had supported a lucrative but otherwise relatively small commercial fishery for many decades (Rowland 1989). Murray cod are undisputedly the largest freshwater fish in Australia with specimens up to 114 kg (1.8 m in length) being recorded (Harris and Rowland 1996).

Murray cod are found naturally throughout the tributaries of the Murray-Darling River system of south-eastern inland Australia. Fish have also been translocated outside their natural range through release of both wild fish and hatchery-produced fingerlings, particularly into farm dams, water storage reservoirs and some river drainages (Harris and Rowland 1996).

The other *Maccullochella* species, trout cod (*M. macquariensis*), eastern cod (*M. ikei*) and Mary River cod (*M. peelii mariensis*) (Table 1.1) are listed nationally as either endangered or critically endangered by the Australian Society for Fish biology (Crook and Pogonoski 2003). However, the status of Murray cod was recently reviewed (Kearney and Kildea 2001) and due to a decline in abundance and distribution in the wild, the species was listed in July 2003 as Vulnerable under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act 1999) (http://www.deh.gov.au/epbc/index.html).

During the 1970's and 1980's techniques were developed that enable routine, relatively large-scale hatchery production of Murray cod (Wyse 1973; Rowland 1983; Cadwallader and Gooley 1985; Rowland 1986b; 1988). State government and private fish hatcheries in NSW, Queensland and Victoria, annually produce fingerling Murray cod for stocking public and private waterways for both recreational and conservation purposes (Rowland 1986b; Gooley 1992a). More recently there has been considerable industry interest (both producers and markets) in the grow-out of Murray cod to satisfy a significant demand for human consumption (Ingram *et al.* 1999; Ingram 2000).

Table 1.1. Summary of known culture information on *Maccullochella* spp.

	Murray cod	**Trout cod**	**Mary River cod**	**Eastern cod**
Species name	*M. peelii peelii*	*M. macquariensis*	*M. peelii mariensis*	*M. ikei*
Farm locations	VIC, SA, NSW, QLD, WA	VIC & NSW	QLD	NSW
Conservation status				
EPBC Act[1]	Vulnerable	Endangered	Endangered	Endangered
ASFB[2]	Not listed	Critically Endangered	Critically Endangered	Endangered
Natural spawning	✓	✗	✓	✓
Induced spawning	✓	✓	✓	✓
Fingerling production	✓	✓	✓	✓
Stock enhancement (as fingerlings)	Recreation/ Conservation Since 1971	Conservation stockings only Since 1986	Conservation stockings only Since 1986	Conservation stockings only Since 1988
Yearling production	✓	✓	✗	✓
Table fish production	✓	✗	✗	✓*

1. Environment Protection and Biodiversity Conservation Act 1999
2. Australian Society for Fish Biology (Crook and Pogonoski 2003)

* Product first sold at Sydney Fish Market in August 2002.

1.2 Commercial fishery

From the late 1800's Murray cod became a significant and valued portion of the commercial inland catch with fish being sold widely in markets throughout south-eastern Australia (Rowland 1989; Kailola *et al.* 1993). Over the past 50 years the fishery has been dominated by fish taken in NSW and SA, though smaller quantities were also taken in Victoria and Queensland (Fig. 1.1a). During the 1950's, the annual catch was mostly over 150 t, and a maximum of 311 t was taken in 1958/59. In recent decades, however, the catch has declined substantially and since the 1960's has rarely exceeded 50 t/annum (Fig. 1.1a).

The value of the commercial fishery, which has been recorded by SA and NSW fisheries authorities since 1984/85, has ranged from $38,000 (1994/95) to $768,000 (2000/01) per annum (Fig. 1.1b). Value per kg over this period has varied from $4.29/kg (1994/95) to $20.95/kg (1998/99) (Fig. 1.1c). In comparison, values for Murray cod sold at the Sydney Fish Market (SFM) (http://www.sydneyfishmarket.com.au/) and the Melbourne Wholesale Fish Market (MWFM) (http://www.chsmith.com.au/fish-prices/melbourne.html) have been variable ($2.5 - $33.6/kg), but are more often between $15/kg and $25/kg (Fig. 1.2a & b). Up to 8.9 t/month and 3.5 t/month have been sold at the SFM and MWFM, respectively (Fig. 1.2c & d).

These figures confirm the relatively high market value of Murray cod, but they also indicate widely fluctuating prices and volumes, which may reflect variations in demand and/or availability of product, as well as quality of product, perhaps associated with capture, handling and transport conditions. The availability of Murray cod at these markets has been variable with product sold two out of approximately

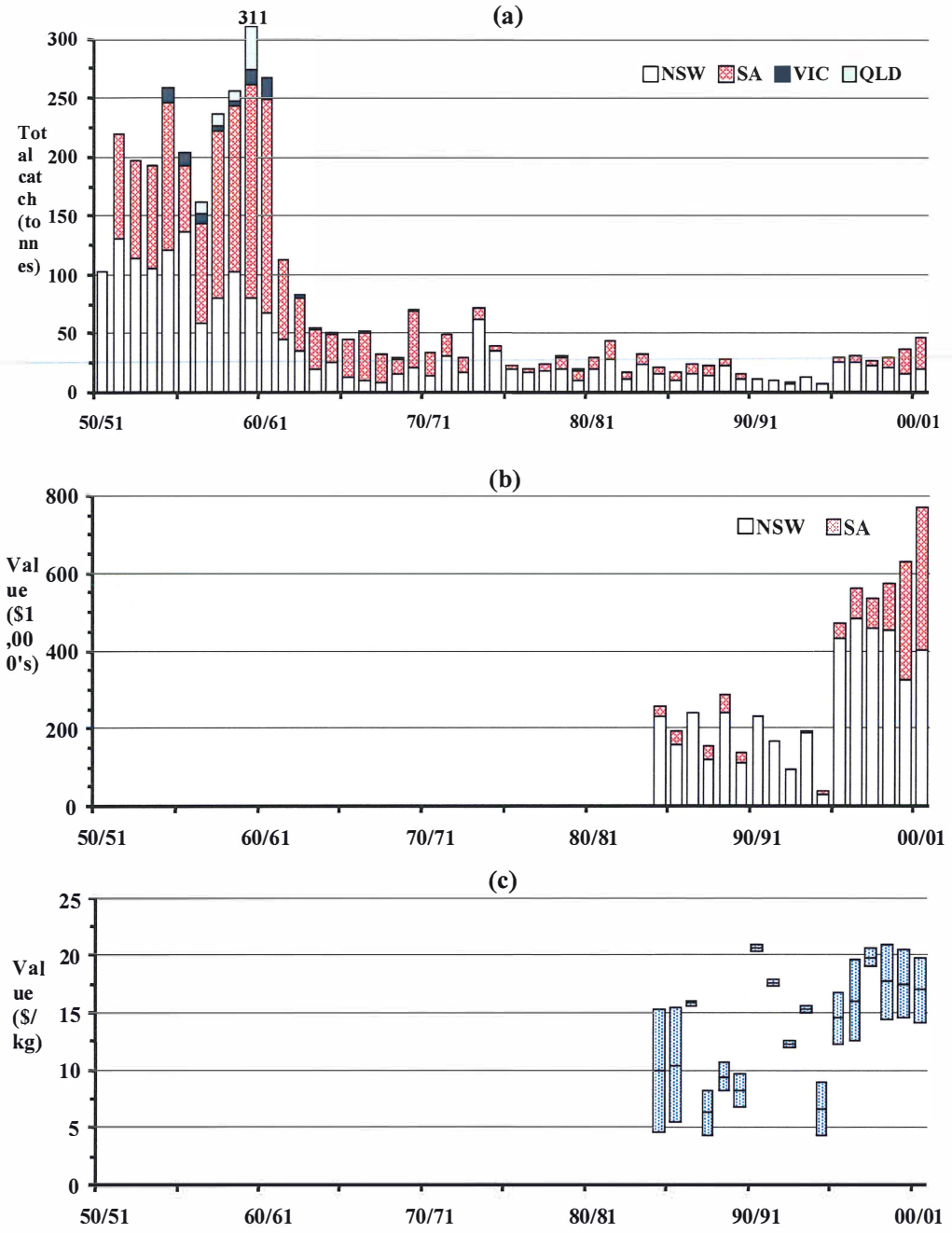

Fig. 1.1. Total annual commercial catch of Murray cod from inland waters of NSW (1950/51-2000/01), SA (1951/52-2000/01), Vic (1954/55-1998/99) and Qld (1956/57-1959/60). (a) Annual catch in tonnes, (b) Value ($1,000's) and (c) Value ($/kg).

Source: *NSW*: NSW Fisheries Commercial Fishing Database.
SA: CBCS (1955-1965) (1954/55-1964/65); Kailola *et al.* (1993) (1965/66-1983/84); Anon (2001a) (1951/52-1983/84), and Knight *et al.* (2002) (1984/85-2000/01).
Vic.: CBCS (1955-1965) (1954/55-1964/65); Kailola *et al.* (1993) (1965/66-1977/78) and Fisheries Victoria (1978/79-2000/01).
Qld: CBCS (1955-1965) (1954/55-1964/65).

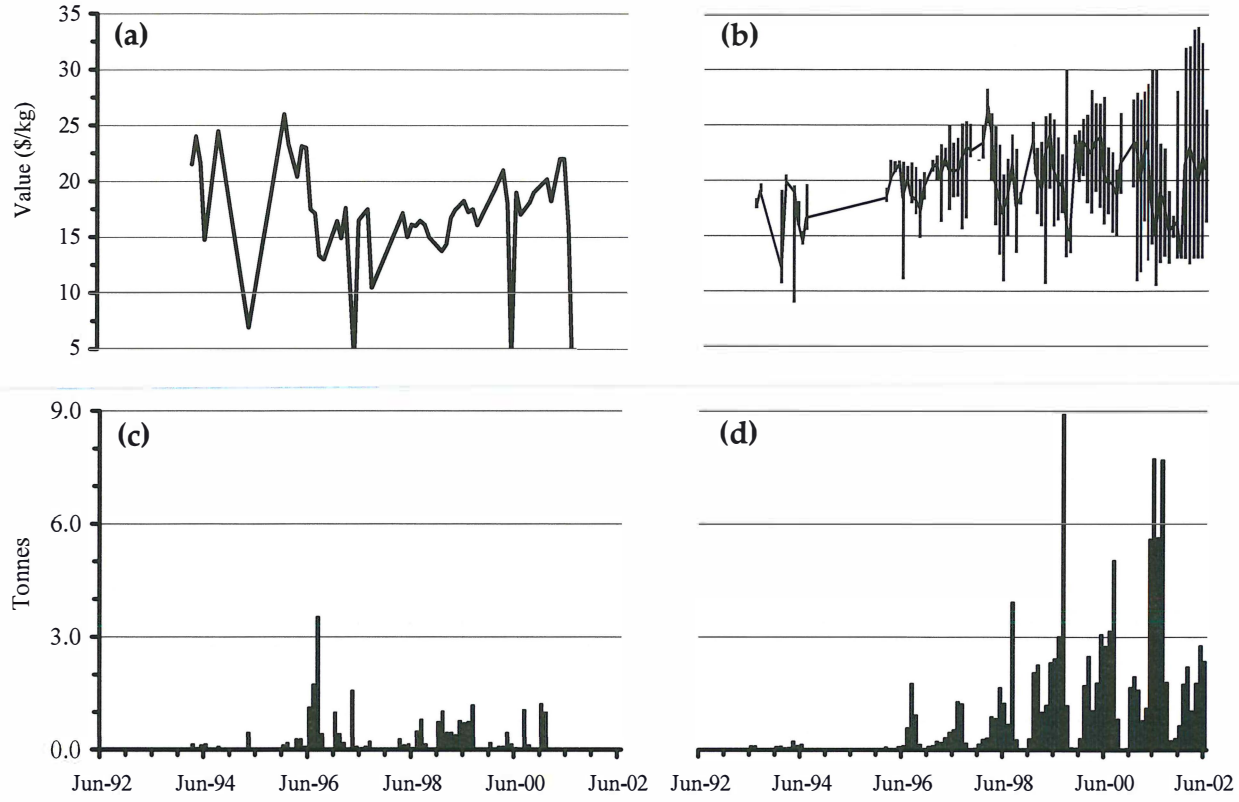

Fig. 1.2. Value and tonnages of Murray cod sold per month at the Melbourne Wholesale Fish Market (MWFM) from March 1994 and the Sydney Fish Market (SFM) from June 1992. (a) Mean value at the MWFM. (b) Mean value (and range) at the SFM. (c) Tonnes sold at the MWFM. (d) Tonnes sold at the SFM.

every three months at the SFM and one out of approximately every two months at the MWFM. In particular, fish are absent from the markets during the months of October and November, presumably due to the affects of a closed season on the take of Murray cod.

Since the 1950's there has been a substantial decline in the distribution and abundance of Murray cod (Rowland 1989). Reasons for this decline are not clear, but contributing factors may have included modifications to rivers for hydro-electric, flood mitigation and irrigation schemes, increased pollution from domestic, agricultural and industrial sources, and over-fishing by both commercial and recreational anglers, and competition with introduced fish species (Cadwallader 1978; Cadwallader and Gooley 1984).

Due to concerns for declining populations of Murray cod in the wild, more stringent management controls have been placed on both commercial and recreational fisheries to help protect the species. Commercial fishing for Murray cod is banned in Queensland and Victoria, and NSW closed commercial fishing for inland native fish, including Murray cod, in September 2001. In South Australia, Murray cod can still be commercially harvested outside the closed season (1 September to 31 December), though this fishery was closed in July 2003 (Anon 2002). In all states where Murray cod occur, recreational fishing regulations include size limits, bag limits and a closed season during the spawning period[1]. The taking of Murray cod by recreational anglers is banned from 1 September to 30 November in NSW, Victoria and parts of Qld, and from 1 September to 31 December in SA. The minimum legal size is 50 cm in NSW, SA and Vic and 60 cm in

1 NSW: http://www.fisheries.nsw.gov.au. SA. http://www.pir.sa.gov.au. Qld: http://www.dpi.qld.gov.au. Vic. http://www.dpi.vic.gov.au.

Qld, and restrictions are imposed on the maximum size of fish that can be taken, which varies from state to state. A bag limit of two fish per day applies in all states.

1.3 Aquaculture

Early attempts to spawn and rear Murray cod were documented by Dakin and Kesteven (1938) and Rowland (1985). In 1905, H.C. Dannevig stripped several wild Murray cod and successfully hatched eggs (Farnell 1906). During the 1960's breakthroughs in the captive spawning of Murray cod occurred at the NSW Government research facility, Narrandera (Lake 1967a; 1967b). Production techniques were further developed at government facilities in both NSW and Victoria during the 1970's (Wyse 1973; Rowland 1983; Cadwallader and Gooley 1985; Rowland 1986b; 1988), and by the early 1980's large-scale hatchery production of Murray cod juveniles was becoming fairly routine at both government and private hatcheries. To this point, most efforts had been focused on the production of fingerlings for stocking purposes. However, by the early 1990's interest in grow-out of Murray cod for human consumption was gathering momentum. By the late 1990's sufficient information had been gathered from various sources (government and university research institutions and private fish farms) to indicate that there was potential for the culture of Murray cod for human consumption.

1.3.1 Spawning

In most cases Murray cod broodfish are held in earthen ponds, which are typically stocked with both males and females in roughly equal numbers. Most broodfish are predominantly wild fish collected from rivers, though some private farms have "second generation" broodfish spawned and reared in captivity. Murray cod are oviparous. Spawning occurs once each year in spring-summer (September-December) and is predominantly triggered by increasing daylength and water temperatures (Harris and Rowland 1996). Induced spawning techniques using injections of hormones followed by hand stripping of gametes have been developed for Murray cod (Rowland 1988). However, most hatcheries currently rely on broodfish spawning unassisted in ponds (Rowland 1983; Cadwallader and Gooley 1985). The eggs (3.0-3.5 mm diameter) of Murray cod, which are demersal and adhesive, are laid on hard surfaces. Specially constructed spawning structures are placed in broodfish ponds to provide a suitable spawning substrate. Spawnings, once detected, are harvested and the eggs are incubated in a hatchery under controlled conditions. At water temperatures of 20-22°C eggs commence hatching 5-7 days after fertilisation and continue to hatch for 3-4 days (Rowland 1983; Cadwallader and Gooley 1985; Rowland 1986b). Newly hatched larvae (Plate 1b) are 5-8 mm in length, and commence feeding about 10 days after hatching is completed (Rowland 1983; Cadwallader and Gooley 1985; Rowland 1986b). In the hatchery the larvae are initially fed on brine shrimp (*Artemia*), though in tank trials, Rowland (1992) showed that at commencement of feeding Murray cod larvae consumed copepodites, copepods and cladocerans, but rarely consumed rotifers.

1.3.2 Fingerling production

Production of fry and fingerlings for stock enhancement programs and for grow-out in aquaculture operations relies to a large extent on the extensive rearing of these fish, stocked as larvae and post-larvae, in fertilised earthen ponds during the first few months of their life. This is a common and widespread practice employed for many species of fish farmed in Australia, including Murray cod (eg. Rowland 1986a; 1986b; 1992). Under extensive pond culture conditions fish are stocked at low densities and there is no supplementary feeding. Instead, naturally occurring aquatic organisms are the sole source of food for fish. Fertilisers are added to the ponds to enhance productivity and encourage plankton growth. Usually, fish are stocked into ponds shortly after exogenous feeding has commenced and when a sufficient amount of plankton of a preferred composition and size range is present in the ponds. At Primary Industries Research Victoria's (PIRVic) Snobs Creek facility (Eildon, Victoria), ponds are usually stocked about 2 weeks after filling and harvested 5-7 weeks following stocking (Plate 1c). At stocking fish are about 15mm in length (<0.1 g) and about 45 mm (1.1 g) at harvest (Plate 1d). Ponds are stocked with up to 25 fish/m^2 and, on average, 75% are recovered at harvest (Ingram 2001).

1.3.3 Stock enhancement

Since the development of mass production techniques in the late 1970's and early 1980's Murray cod have been widely stocked into rivers, farm dams and impoundments, for conservation, stock enhancement and recreational purposes (Rowland *et al.* 1983; Gooley 1992a). More specifically, reasons for stocking Murray cod have included establishment of recreational fisheries in man-made impoundments, re-establishing and enhancing populations in areas where the species has declined or become extinct, and stocking of farm dams on private properties. The vast majority of fish, however, are being stocked into public waterways, mainly man-made impoundments, reservoirs and weirs, but also rivers and streams.

The first state government-funded stockings of hatchery-produced Murray cod into public waters occurred in 1971/72 in NSW and 1980/81 in Victoria when 1,270 and 6,000 juveniles were released, respectively. Since then the number of Murray cod released annually has steadily increased. In the late 1990's between 500,000 and 1.1 million fish were released annually (Fig. 1.3). Stocking of private farm dams in south-eastern Australia has also occurred throughout this period. Since the commencement of stocking and up to the 2001/2002 season, about 6.9 million fish have been released. To meet this demand, government hatcheries in NSW and Victoria, and private hatcheries in NSW, Victoria, and Queensland routinely produce large numbers of fingerlings. The majority of fish that are released are fingerlings (0.75-1.5 g, 40-55 mm) that have been reared in fertilised fry rearing ponds. However, some stockings, particularly in the early years of captive breeding of the species, were undertaken with post-larvae and fry (10-20 mm) (eg. Gooley 1992a). Since the development of techniques to grow-out Murray cod, small numbers of yearling Murray cod (up to 300g) have also been released in recent years in both NSW and Victoria. The majority of stockings are being undertaken by state governments with fish produced at government-owned hatcheries in NSW (Narrandera) and Victoria (Snobs Creek), as well as with fish purchased from private hatcheries. Recreational angling groups are also releasing large numbers of fish, purchased from both government hatcheries and private hatcheries in NSW, Victoria and Queensland. Most stockings (>85% of fish released) have occurred into waters within the Murray-Darling Basin in NSW and Victoria (Fig. 1.3). This is not surprising considering that most of the natural range of Murray cod falls within these two states. Smaller numbers of fish have also been released into waters in the ACT and Queensland (Fig. 1.3). Yet, despite the occurrence of Murray cod, no meaningful stockings have occurred in South Australia. Small numbers of juvenile Murray cod are also being purchased by individuals for stocking of private waters, such as farm dams. Some waters outside the natural range of Murray cod have also been stocked with both hatchery-produced fish and translocated wild fish (Cadwallader and Gooley 1984; Harris and Rowland 1996).

Currently, fingerlings are usually available only during production season, with stockings occurring mostly between December and May. Prices of fingerlings range from about $0.45-0.60/fingerling for lots of >10,000, and up to $1.60/fingerling for small consignments.

1.3.4 Grow-out

Since the early 1990's there has been considerable industry interest in the grow-out of Murray cod to satisfy a significant demand for human consumption, in both domestic and international markets (Ingram 2000). Such a demand is in part driven by the premium and associated ongoing demand in the domestic market for Murray cod, and in Asian markets for marine groupers and similar perch-like fishes (eg. the freshwater Mandarin fish in China). There is a perception that Murray cod are very similar in appearance to these premium species (Anon 2001b).

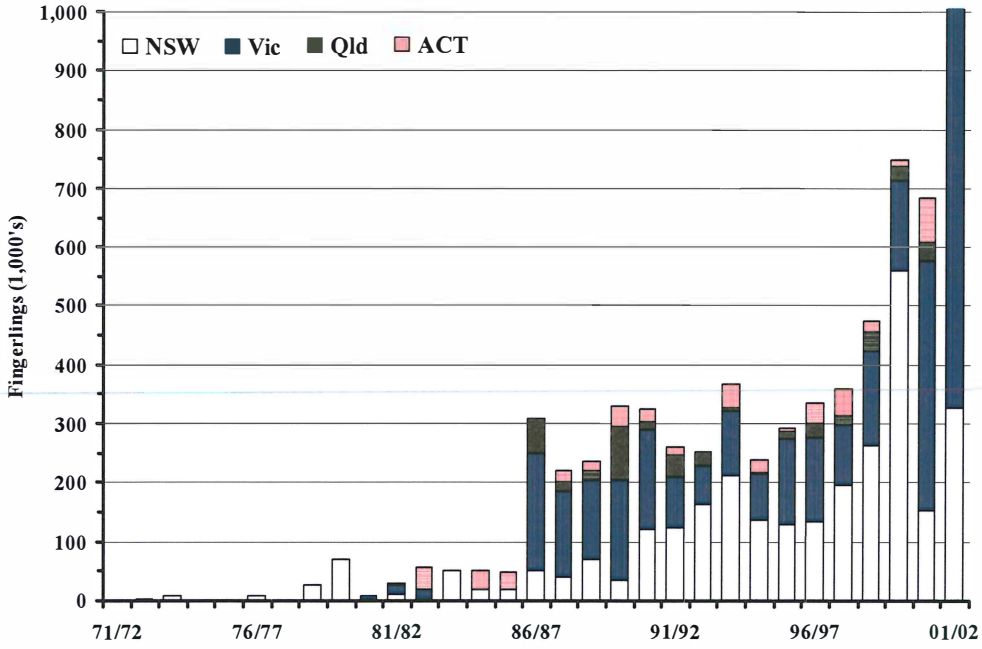

Fig. 1.3. Numbers of hatchery-produced juvenile Murray cod released annually for stock enhancement purposes in Victoria, NSW, Queensland and the ACT since 1971/72.

Source Victoria. Fisheries Victoria and PIRVic, Snobs Creek; Includes fish released by both government and client groups. Includes fish stocked as fry (10-20 mm), fingerlings (40-55 mm) and yearlings (75-150 mm).
NSW. NSW Fisheries. Draft figures only. Includes fish released by both government and client groups. Includes fish stocked as fry, fingerlings and yearlings.
Queensland. Department of Primary Industries, Queensland.
ACT. Wildlife Research and Monitoring, Environment ACT.

Until the current study, information on the growth of Murray cod was generally limited to studies of wild fish, and mostly sub-adult and adult fish. Length-weight and age-weight relationships have been published for wild populations of Murray cod (Anderson *et al.* 1992; Gooley 1992b; Rowland 1998a). Few studies have reported on the grow-out of Murray cod in ponds (eg. Barlow and Bock 1981; Cadwallader and Gooley 1985). Barlow and Bock (1981) found that the growth of juvenile Murray cod stocked into farm dams as fingerlings was highly correlated with temperature, and size range became increasingly pronounced with time. Forster (1999) indicated that 1g Murray cod stocked into farm dams take three years to reach a size of 2-3 kg. In both of these studies, fish were stocked at low densities (<500 fish/ha) and received little or no supplementary feeds. Cadwallader and Gooley (1985) described preliminary observations on the on-growing of juvenile Murray cod held in aquaria, tanks and swimming pools. Although fish were successfully reared for up to one year, Cadwallader and Gooley (1985) suggested that large scale on-rearing was not practical due to behavioural interactions (aggression, territoriality and cannibalism) that limited stocking densities, stress induced diseases and the amount of maintenance require to maintain systems. Cage culture of Murray cod has not been reported in recent times, but Dakin and Kesteven (1938) described a trial in 1937 in which Murray cod that were held in a cage (3 x 0.9 x 0.6 m) for over 13 weeks readily accepted hand feeding with mussel flesh.

Some private fish farms have begun to commercially produce edible or market-size Murray cod in tanks and ponds with both natural and artificial diets under a range of intensive/semi-intensive and ambient/controlled environmental conditions. Early results from intensive tank culture trials indicate potentially excellent growth rates with 2g fish reaching a plate size of 600g within 12 months (at a temperature of 25°C), with survival rates in excess of 80% and stocking densities at 80-100 kg/m^3 (Ingram *et*

al. 1999). Much of these initial trials were being undertaken with feeds formulated for other species (eg. salmonids, barramundi etc). Preliminary investigations have been undertaken into nutritional requirements and development of artificial diets for Murray cod (Anderson and Gooley 1992; Gooley and Anderson 1992; Gunasekera *et al.* 1999), but specific formulated diets for the rearing of juvenile and sub-adult Murray cod are not yet commercially available. Although Murray cod are amenable to a wide range of environmental conditions, water quality requirements for optimal production of Murray cod in culture systems are not known. A range of health problems and diseases have been identified in Murray cod (eg. Rowland and Ingram 1991), but the extent to which these will inhibit commercial production of Murray cod is unclear.

Production figures reported for table fish in the early 1990's are somewhat erroneous as few/no farms were in production. But by the mid-1990's small amounts (<1 t/annum) of farmed Murray cod were appearing in aquaculture production statistics (O'Sullivan and Kiley 1996; O'Sullivan and Kiley 1997), and by the late 1990's annual production had increased to over 30 t/annum (O'Sullivan and Dobson 2000). Farm gate prices being paid for farmed Murray cod during the late 1990's were in excess of $20/kg (Larkin and Ingram 2000; O'Sullivan and Dobson 2000).

1.4 Aquaculture attributes of Murray cod

There are various key biological and economic factors that are commonly highlighted as indicators of a species' suitability to intensive commercial aquaculture (Bardach *et al.* 1972; Webber and Riordan 1976; Shepherd 1988; Pillay 1990; Rowland and Barlow 1990). The following is a brief annotated checklist of the key biological and economic attributes which indicate the suitability of Murray cod as a candidate for intensive commercial aquaculture.

1.4.1 Biological attributes

- *Breed easily under captive conditions.* Breeding techniques for Murray cod are well established, with hatcheries in NSW, VIC and Qld routinely mass-producing fingerlings from captive broodstock (see previous).
- *High fecundity.* Murray cod has a relatively low fecundity, producing 3,200-7,600 eggs/kg (Rowland 1998b), when compared to other well-recognised aquaculture species (silver perch – 250,000-300,000 eggs/kg, African catfish – 80,000 eggs/kg, common carp – 150,000 eggs/kg) (Merrick and Schmida 1984; Bromage 1988). However, species that produce fewer and larger eggs, tend to have larger and therefore more hardier larvae, as is the case with Murray cod and several successfully farmed species, such as rainbow trout (2,200 eggs/kg) and channel catfish (7,000 eggs/kg) (Bromage 1988).
- *Readily accept artificial feeds from a young age.* Murray cod are active, capable feeders at the completion of yolk sac absorption, and can be readily weaned onto artificial feeds at a young age (Ingram and Larkin 2000).
- *Low trophic level feeder (herbivorous, omnivorous).* Fish that can utilise plant matter can be reared relatively cheaply. These species can feed on algae and phytoplankton in the culture environment and may require supplementary feeding on artificial diets only. Artificial feeds for these species are more cost effective as less fishmeal (a major cost component of feeds) is required. Murray cod is a carnivore (Harris and Rowland 1996) and by comparison, such species are generally more expensive to produce as they usually require a diet with a high protein content. To compensate for feeding costs, farmed carnivorous species must command a higher market price than other species, which Murray cod does.
- *High rate of growth and production under culture conditions.* Early results from industry suggest that Murray cod will grow from 2g to 600g in 12 months (water temperature of 23-25°C and on sub-optimal diets; survival >80%) (Ingram *et al.* 1999).
- *Good feed conversion efficiencies throughout the production cycle.* At this stage, there are no reliable data on food conversion efficiencies for Murray cod.
- *Reach market size before reaching maturity.* This means that feed and energy are used for somatic growth rather than gonad development. Murray cod reach maturity in the wild at between 4-5 years (Harris and Rowland 1996). Initial observations from the aquaculture industry indicate that Murray cod reach a market size of 600 g in less than 15 months, well before any significant sexual maturation takes place.
- *Hardiness and adaptability to crowding in confined spaces* (as experienced in intensive culture conditions). Have broad water quality requirements, have favourable behavioural traits (non-territorial, non-cannibalistic) and have good disease resistance. Preliminary information from industry indicate that

Murray cod can be stocked at high densities (80-100 kg/m^3) and can be easily handled for grading purposes (Ingram *et al.* 1999). However, the water quality requirements for optimal production of Murray cod under commercial conditions were not well known. Murray cod are reported to be aggressive, territorial and cannibalistic in nature (Cadwallader and Backhouse 1983; Cadwallader and Gooley 1985). This is not a favourable traits as they can reduce production through lowered survival and growth due to predation and stress-related factors associated with attack by larger fish.

- *Disease resistance.* Murray cod are susceptible to some common fish diseases both under culture conditions and in the wild (Rowland and Ingram 1991).

1.4.2 Economic attributes

- *Consumer acceptance, demand and availability of markets.* Murray cod is a highly prized recreational species, which is valued for its sport and edibility. The fillets of Murray cod are white, firm with a medium to high fat content and a distinct, delicate flavour. Taste testing by seafood experts specialising in Asian markets indicated that Murray cod has an appropriate fat content, good texture and colour, good meat recovery and minimal bones (Anon 2001b).
- *Market value.* There is an existing domestic market for Murray cod that up until recently was based on a small wild (<50 t) commercial fishery that commenced in the late 1800's (Fig. 1.1). Murray cod is a premium-priced fish at both the Melbourne and Sydney fish markets where during the late 1990's prices were generally between $15/kg and $25/kg for mainly wild-caught fish (Larkin and Ingram 2000). Whether or not these prices can be sustained for cultured product, and more than compensate for relatively high production costs has yet to be determined for Murray cod. A Bio-economic model (AQUAFarmer^{TM2}) has been developed by Fisheries Victoria to assist existing and prospective Murray cod farmers to assess economic performance.

1.5 Technical constraints, information gaps and R & D needs

Problems encountered to date in the aquaculture of Murray cod have included:

- A lack of basic husbandry guidelines at all levels, including hatchery, nursery and grow-out.
- Difficulty in sourcing and weaning good quality seedstock, especially with increasingly competing demands for stock enhancement purposes. The lack of rigour in sourcing and managing broodstock is presently aggravating this problem.
- Inadequate culture systems with insufficient performance criteria for effective economic evaluation. This information is required to evaluate profitability by comparing cost of production against market prices.
- A lack of understanding of baseline environmental requirements (temperature, water quality, light etc.) for optimising intensive production. More specifically, although Murray cod are amenable to a wide range of environmental conditions, specific conditions in culture systems for optimal intensive production performance (growth, FCR and survival) have yet to be identified.
- A lack of properly formulated, commercially available artificial diets.
- A lack of a clear market direction/strategy, and associated quality assurance, for cultured Murray cod.
- A lack of relevant business models upon which commercial investment in Murray cod aquaculture can be based.
- A lack of an appropriate industry development model to facilitate commercial investment.

Apart from these issues, the overall economic viability of commercial scale, high density grow-out aquaculture for Murray cod is yet to be determined, as indeed is any evaluation of the optimal culture system and associated environmental conditions and production support parameters that will be necessary for long term industry investment and market confidence. In the Australian domestic market, potential exists for achieving a minimum 100-200 t/$2-4 million annual production with existing producers alone within 3-5 years, and there is significant potential for export into Asian markets (Anon 2001b). However,

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2 *AQUAFarmer (V2.1)* is propriety software developed by Fisheries Victoria. It is specifically designed to analyse recirculation aquaculture efficiency and viability. It is a 10 year accounts simulator that creates farm scenarios based on bio-economic inputs.

despite these apparent market opportunities, there is a specific need to undertake a formal market appraisal of domestic and/or export opportunities for commercially cultured Murray cod and associated value-added products.

1.5.1 R&D Needs

Specific industry needs for Murray cod R&D were identified via consultation with government and industry. On 13 August 1998, PIRVic convened a meeting with commercial warmwater fish farmers, predominantly from Victoria and Southern NSW, and representatives from Fisheries Victoria, Deakin University and the Victorian Institute of Animal Science. This meeting discussed research needs and established a network of Murray cod farmers willing to collaborate on projects. In March 1999, a detailed questionnaire was circulated to a selection of existing and/or proposed Murray cod farmers in Victoria, SA and NSW, to further identify industry R&D needs. The results of the questionnaire were collated and summarised and the four highest ranked specific R&D priorities (accounting for 63% of the total vote) were:

- *Fish health*: Minimising stress from outbreaks and therapeutic treatments to maximise not only survival but also long term growth is critical. Disease induced checks to growth at key physiological development stages has profound impacts on future production. For example, up to 30% loss of available seed can occur during the weaning/immediate post-weaning phase due disease induced mortalities.
- *Diet development*: Increased production through intensification requires nutrient dense (high energy) feeds and more efficient feeding practices. Artificial diets currently in use for this purpose are largely adapted from existing salmonid, barramundi and silver perch diets, and are not specific formulated for Murray cod. Consequent problems include sub optimal FCR's and developmental problems such as "fatty liver".
- *Genetic improvement*: Most broodstock currently in use for seedstock production are essentially selected from wild populations and/or first generation (F1) progeny randomly selected from hatchery fish. The breeding system is defined as an "open" system in which there is no/little effort to select hatchery progeny as future broodstock based on specific characteristics suitable for specific markets. Indeed, to date considerable effort has been extended in some hatcheries to ensure genetic integrity remains intact for enhancement of wild populations by maximising/randomising genetic resources. Selection for improved growout performance at the same time as protecting wild genetic material is the imperative.
- *Market information and bio-economic modelling*: Despite the apparent market opportunities for farmed Murray cod, based largely on anecdotal information, there is a specific need to undertake a formal market appraisal of domestic and/or export opportunities for commercially cultured Murray cod and associated value-added products. From such information and cost benefit data, a simple but reliable interactive pc-based model for analysis of economic viability of Murray cod farming systems can be developed.

1.6 The present study

The present project entitled *Development of Intensive Commercial Aquaculture Production Technology for Murray Cod* (FRDC Project No. 1999/328) was developed to address some of the technical constraints, information gaps and R&D priorities identified by industry and to facilitate industry development of Murray cod aquaculture. Note that, despite being identified as an R&D priority, genetic improvement of Murray cod for aquaculture purposes was deemed outside the scope of the project due to lack of available resources. The project was jointly funded by PIRVic (then MAFRI), the Fisheries Research and Development Corporation (FRDC), Fisheries Victoria and Deakin University, Warrnambool, with in-kind support from industry (including fish farmers, feed manufacturers and system retailers). Actual production trials and associated experimentation were undertaken at a combination of locations including PIRVic (Snobs Creek), Deakin University Warrnambool and *in situ* at commercial farms. The project was managed by PIRVic, Snobs Creek. The project commenced in July 1999 and was scheduled to be completed in June 2002.

1.6.1 Project objectives

(a) To develop and evaluate Best Practice husbandry, nutrition and fish health for commercial production of Murray cod under extensive pond-based hatchery, and intensive tank-based growout conditions.

(b) To develop and implement an appropriate extension and associated market strategy to ensure effective and efficient transfer of research outcomes and associated protocols and technologies to industry.

Key tasks of the present project included to:

- Validate and refine existing and/or developing new husbandry techniques pertaining to the culture of Murray cod.
- Design and undertake diet formulation and testing investigation for juvenile and sub-adult Murray cod.
- Investigate the fish health implications of high-density aquaculture of Murray cod.
- Undertake a survey of market opportunities for commercially cultured Murray cod and associated value-added products and to provide baseline cost-benefit analysis for developing models for analysis of economic viability of potential Murray cod culture systems.
- Undertake two industry workshops for dissemination of information to industry resource/policy managers and other researchers.

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2 MURRAY COD AQUACULTURE: INDUSTRY STATUS

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CONTENTS

2.1 Introduction

The techniques for large scale Murray cod (*Maccullochella peelii peelii*) fingerling production have been developed over the last 30 years, with fingerlings traditionally produced for restocking public and private waters. In recent years there has been a trend towards weaning and on-growing fingerlings commercially for human consumption, not just within Australia but also overseas.

Murray cod, by its nature being a large, aggressive, carnivorous, territorial species, would traditionally not be considered as an ideal aquaculture species. However, Murray cod has a number of attributes that make it suitable for aquaculture. These include:

- breeds easily in captivity
- accepts artificial feeds
- high rate of growth and production in aquaculture (2-600 grams in 6-14 months at 20-25°C)
- reaches market size before maturing
- hardy and adaptable to crowding (routinely cultured at 80-100 kg/m^3)
- highly valued species that is well known in the markets.

Production of Murray cod for the table fish market is a relatively new industry, with on-grown fish entering the markets for the first time in the early 1990's (Gooley and Rowland 1993). Since then, this sector has grown considerably. In 2000, when the first review of the Murray cod aquaculture industry was undertaken (Larkin and Ingram 2000), six farms only had apparently on-grown and sold small volumes of table size Murray cod. However, at that time interest in aquaculture of this species for grow-out was substantial, not only by aquaculturists that were producing other species, but also by potential new investors. The aim of the present study was to update the status of the Murray cod aquaculture industry and present information

on the Murray cod Aquaculture Network (MCANet), fingerling and table fish production levels and industry value.

2.2 The Murray cod Aquaculture Network (MCANet)

As part of the project, a network of persons interested in Murray cod aquaculture, the Murray cod Aquaculture Network (MCANet), was established to provide a means of information exchange and dissemination of results and information from the project. Currently, MCANet has 244 entries with representation from industry (farmers/producers, industry associations, consultants, investors, feed manufacturers, equipment suppliers etc) (49%), government (19%) and education (universities, TAFE etc) (4%) (Fig. 2.1). Half (50%) of the entries are from Victoria, followed by NSW (21%) and Queensland (13%), with the remaining from other states (15%) and overseas (1%) (Fig. 2.1). Of those represented by industry, 68% are from operating fish farms, (65% of which have Murray cod on-site) and 20% are providers (aquaculture equipment suppliers, feed manufacturers etc).

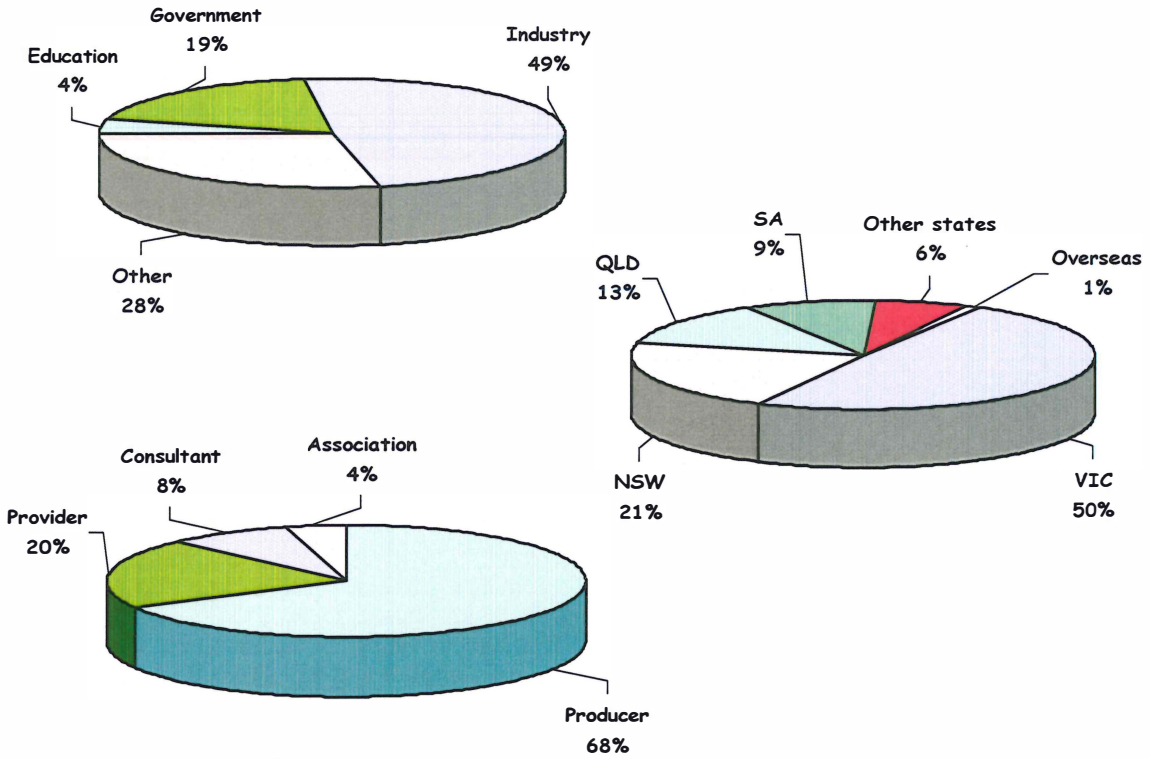

Fig. 2.1 Make-up of the Murray cod Aquaculture Network (MCANet).

2.3 Industry demographics

Interest in the farming of Murray cod is substantial across much of Australia and even overseas. In January 2000, about 30 farms had Murray cod on site, which included, broodfish, juveniles (fingerlings etc) and market sized stock (Larkin and Ingram 2000) (Fig. 2.2). By August 2002, this number had doubled with in excess of 60 farms with Murray cod on site. The number that reported to be producing seedstock (fingerlings) increased from 7 to 19 over the same period. A total of six farms had grown and sold marketable fish (table-size fish) in January 2000, but this figure had increased to 19 by August 2002 (Fig.2.2). The level of production grow-out farms has ranged from < 1 t/annum/farm to over 100 t/annum/farm.

In January 2000, most production was confined to Victoria, NSW, SA, but by August 2002, production was also occurring in Queensland and more recently in WA (Fig 2.2). In addition, there are numerous fish farmers in at least some of these states that are licensed to produce Murray cod, but have yet to do so.

Most Murray cod farms (approximately 66%) are located within the Murray-Darling Basin, reflecting the natural distribution of the species. All production of Murray cod fingerlings, which includes the use of open ponds for holding broodfish and rearing juveniles, occurs solely within the Murray-Darling basin. However, use of bio-secure, recirculating aquaculture systems (RAS) has allowed for the grow-out production of Murray cod outside their natural range. There are both national and state guidelines/policies controlling the translocation of live aquatic organisms (MCFFA 1999), which also apply to species being farmed outside their natural range. These guidelines typically entail an impact risk assessment of the proposed translocation being undertaken. Risk management measures put in place usually involve the development of bio-secure conditions for culture of the species and often these can best be achieved in RAS. For example, in Victoria, a number of small RAS that grow Murray cod are located on the coastal seaboard near to Melbourne. As part of licensing requirements, these farms must comply with specific "bio-secure" conditions including, that seedstock be health certified, farms are located 500 m or further away from a waterway or irrigation canal, be above the 1-in-100 year flood level, the growout facility be housed in a fully enclosed facility with a concrete floor and bunding of sufficient height to hold the total volume of water under culture to prevent accidental release of water and stock, and that all water outlets be screened to prevent escape of stock (Larkin and Ingram 1999).

Interest in the culture of Murray cod has extended overseas. Australian native finfish are apparently held in high esteem in south east Asian countries, and by the early 1990's, shipments of live silver perch and golden perch fingerlings had been exported to China, whereas few, if any, Murray cod fingerlings had been exported (Gooley and Rowland 1993). However, large numbers of Murray cod fingerlings have since been shipped to several countries in south-east Asia, including Taiwan and China (eg. McGinty 2002).

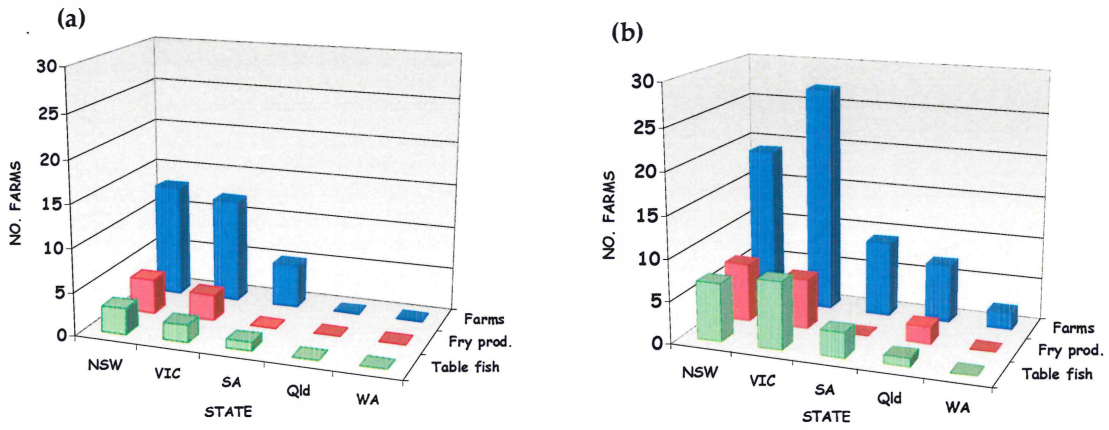

Fig. 2.2. Number of fish farms involved in the culture of Murray cod. (a) January 2000 (source Larkin and Ingram 2000). (b) August 2002 (source: MCANet, and State Aquaculture Extensions Officers). Farms = number farms with Murray cod on-site. Fry prod. = farms producing seedstock (fry & fingerlings). Table fish = Farms selling table-size fish.

2.4 Production and value

2.4.1 Fingerling production

Large-scale commercial production of Murray cod fingerlings was developed during the late 1970's and early 1980's (Ingram *et al.* 2004a). Most fingerlings are produced from the natural spawning of broodfish held in earthen ponds. Once exogenous feeding has commenced, post-larvae are stocked into shallow earthen ponds for grow-out (Plate 1c). These ponds are fertilised to encourage the growth of plankton and other aquatic organisms on which the fish feed (Rowland 1986a; 1986b; 1992). At harvest from these ponds, fingerlings are typically about 45 mm in length and about 1 g in weight (Plate 1d). Fingerlings are usually

available between December and May, which reflects the seasonal nature of current production methods. Initially fingerlings were produced to support stock enhancement programs (Ingram *et al.* 2004a). However, since the mid 1990's when farms commenced on-growing Murray cod for the table market, fingerlings are now also being produced to meet the rising demand from this industry sector.

Government hatcheries in NSW and Victoria and private hatcheries in NSW, Queensland and Victoria are producing Murray cod fingerlings. Levels of fingerling production have increased steadily since the 1980's. Between 200,000 and 800,000 fingerlings were produced annually during the 1990's, and in the 2000/2001 season a record 1.79 million fingerlings were produced (Fig. 2.3). The largest fingerling producer is NSW (50-70% of total production), followed by Victoria then Queensland.

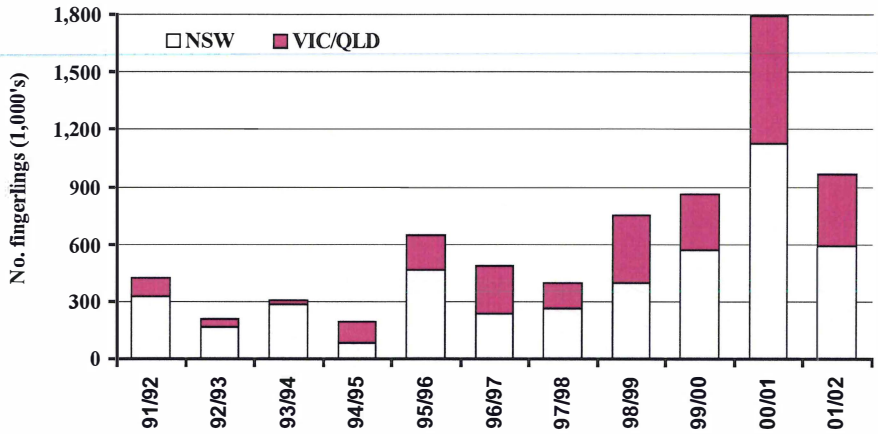

Fig. 2.3. Hatchery production of Murray cod fingerlings from 1991/92 to 2001/2002.

Source: State Government Fisheries aquaculture production statistics.
Note: Data combined for Victoria and Queensland due to the small number of farms. Information from Queensland includes small numbers of Mary River cod and sleepy cod for some years

Fingerlings produced by government hatcheries are principally for stock enhancement programs only, whereas private hatcheries produce fingerlings for both stock enhancement and grow-out purposes. In January 2000, approximately 25% of fingerling production was being on-sold to other farms for grow-out purposes (Larkin and Ingram 2000). Based on commercial aquaculture statistics collected by state fisheries authorities and conversations with Murray cod hatchery operators, currently about 50% of fingerlings are now being sold to other farms for grow-out purposes. This is supported by observations in Queensland where it is reported that approximately 50% of Murray cod fingerlings produced in 2000/01 were sold to other aquaculture operations (Lobegeiger 2002). Many fingerlings are sold interstate, such as into SA where no fingerling production occurs.

Methods for weaning of Murray cod fingerlings onto artificial diets have been described (Ingram *et al.* 2001). Some hatchery operators are undertaking the weaning of fingerlings prior to sale (Mosig 2002), but some grow-out operators prefer to purchase non-weaned fish and undertake the weaning process themselves.

The demand for fingerlings is high and availability is affected by several factors. Spawning occurs once each year in spring-summer (September-December), which means that fingerlings are available seasonally only (December-March). Levels of production vary from one season to the next (eg. Fig. 2.3), which may be associated with the size/maturity, number and condition of broodstock, as well as other factors that might influence spawning such as year to year variations in environmental conditions and changes in water quality. There is strong competition for fingerlings from stock enhancement programs. The number of Murray cod fingerlings being released annually for stock enhancement purposes has increased considerably over the last 10 years and currently between 500,000 and 1 million fish are being stocked each year (see Fig 1.3) (Ingram *et al.* 2004a). This means that often both existing and new Murray cod farmers need to advance

order seedstock to ensure supply. According to members of the MCANet, many hatchery operators have been increasing broodfish numbers of culture facilities to help meet this demand. One possible solution to this problem is the development of "out-of-season" spawning of Murray cod using broodfish held in environment control systems. Although captive Murray cod broodfish have already been successfully spawned under controlled environment conditions at Primary Industries Research Victoria's (PIRVic) facilities at Snobs Creek (G.J. Gooley *pers comm*), levels of fingerling production and associated bio-economic benefits that can be achieved by this method have yet to be determined. Similarly, bio-economic analyses will need to be undertaken to determine the effect of a mid-season intake of seedstock, as opposed to a single intake of seedstock during the normal season (December-March), on production in intensive grow-out facilities.

2.4.2 Table fish production

The vast majority of grow-out from the fingerling stage to market size is occurring in recirculating aquaculture systems (RAS) (Plates 1e & 1f). These systems enable environmental control and if designed correctly enable optimum stocking densities to be for culture. RAS typically involve high installation and operating costs and require a species that will achieve maximum returns or of high market value (see Larkin 2000; O'Sullivan 2000a; 2000b; Rawlinson 2004). Currently, cost of production in RAS varies but is expected to be between $7–10/kg. Operators emphasise the need to have experience in fish husbandry and an understanding of water quality parameters in the management and operation of RAS.

RAS used for the culture of Murray cod vary considerably in design, configuration and size, with levels of production from <1 t/annum to over 120 t/annum. Fish holding capacity (resident stock) of these systems is about 1/3 to 1/2 the annual level of production. The amount of water that is replaced in the system depends on water availability and system design, and may vary from 10% to 50% of the total volume daily. Culture temperatures in RAS are commonly between 20°C and 25°C. Stocking densities employed are highly variable. In aerated systems, densities rarely exceed about 80 kg/m^3, whereas in systems that use oxygen (generated on-site in bulk) densities are usually between 60 and 200 kg/m^3, but densities in excess of 280 kg/m^3 have been reported (O'Sullivan 2003). In RAS, Murray cod generally reach 500-800 g in less than 6-14 months (O'Sullivan 1999; O'Sullivan and Ryan 2002; O'Sullivan 2003).

Currently, there are no commercially available artificial diets specifically formulated for Murray cod. Farmers are therefore using commercial extruded salmonid (rainbow trout or Atlantic salmon) and/or barramundi diets. Both floating and sinking pellets are being used. Feed rates vary depending on size of fish and culture water temperature. Food conversion ratios of between 1.2 and 1.5 are being attained.

Murray cod can be aggressive, territorial and predatory in nature, and cannibalism can occur in culture tanks where densities are low and/or the size range of fish is great. Stress associated with cannibalism may reduce growth rates in smaller fish and lead to increased susceptibility to disease. Therefore grading occurs regularly (every 2-4 weeks) especially in the first few months of grow-out. Some farmers believe that increasing stocking densities may reduce the ability of fish to establish territories and thereby reduce the impacts of territoriality and aggression.

Some farmers are utilising earthen ponds in their grow-out operations. Forster (1999) has stocked 1 g Murray cod (300-500 fish/ha) into earthen ponds under ambient conditions where they fed on naturally occurring prey items (yabbies, shrimp and aquatic insects). These fish reached a market size of 2-3 kg in 3 years.

Alternatively, some farmers are "overwintering" stock in RAS under controlled environment conditions then "finishing-off" stock in earthen ponds (eg. Mosig 2000). Fish are initially stocked into a RAS for grow-out under intensive conditions over winter, taking advantage of the elevated water temperatures that can be achieved with a RAS. At the onset of spring, fish are then moved into earthen ponds and on-grown to a market size under extensive or semi-intensive ambient conditions. Fish that are stocked into ponds at a size of 250-500 g (2-2.5 t/ha) and reach a market size of 1.5-3 kg (up to 15 t/ha) by the end of summer. Some grading and culling (by selective netting) is undertaken during this period. To date there has been no commercial production of Murray cod in cages, however, trials conducted at PIRVic suggest that this culture method is feasible for the species (see Ingram 2004).

Fish are being sold at a size from 600 g to about 2 kg (Plate 2d). Most fish are sold either live, fresh on ice (whole or gilled and gutted) (Plate 3b), or as fresh chilled fillets. Product is being sold through fish markets, wholesalers, distributors, restaurants as well as direct to consumers (Larkin *et al.* 2004).

Most operators are "purging" market-size fish for up to 2 weeks in "clean" filtered water to eliminate taints or off-flavours (muddy/earthy flavours) from the flesh (eg. O'Sullivan 2003). The presence of these taints can render the fish unacceptable to consumers, and can severely reduce their market value (Larkin *et al.* 2004). Fish reared in both RAS and ponds require purging. Fish produced in RAS are often much darker in appearance than wild Murray cod, and may be less attractive to some buyers. When Murray cod that have been reared in a RAS are transferred to ponds, their coloration reverts to that more typical of a wild Murray cod. This change in coloration has been observed in cage culture trails at PIRVic Snobs Creek (see Ingram 2004).

Annual production of Murray cod has grown exponentially over the past five years (Fig 2.4). During the mid to late 1990's production was less than 15 t/annum, but by the turn of the century, production had increased to in excess of 50 t/annum and in the 2001/2002 season 103 t were produced (Fig. 2.4). Annual production statistics indicated that 11 t of farmed Murray cod was produced in 1991/92 season (O'Sullivan 1994), however, this value is considered erroneous and not included here. Since 2000/2001, more than 90% of the total annual production of Murray cod occurred in Victoria (Fig. 2.4), while the balance of production occurred predominantly in NSW.

2.4.3 Industry value

The total value of the Murray cod aquaculture industry (hatchery production of fingerlings and table fish production combined) has increased significantly, from less than $400,000/annum during the 1990's to $2.48 million/annum in 2001/2002 (Fig 2.5). Between 1994/1995 and 2001/2002 the hatchery production sector was valued at $160,000-$970,000/annum (mean $371,000/annum). Between 1994/1995 and 2001/2002 the value of grow-out sector (table fish production) increased from nil to $2.0 million/annum. During the 1990's, the total industry value was predominantly from production of fingerlings whereas in latter years, an increasingly higher proportion of the industry value was derived from the grow-out sector. In 2001/2002, the grow-out sector represented 80% of the total industry value (Fig. 2.5).

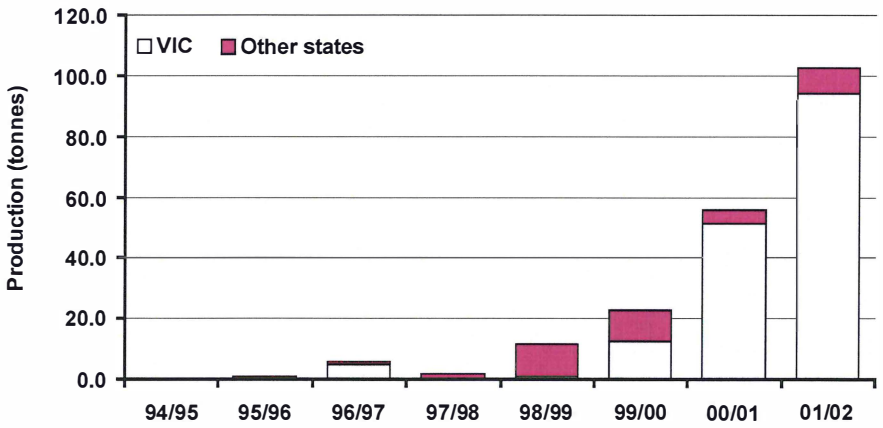

Fig. 2.4. Annual production of farmed Murray cod from 1994/1995 to 2001/2002.

Source: State Government Fisheries aquaculture production statistics.

Note: Data combined for Queensland, NSW and SA due to the small level of production and number of farms in Queensland and SA. Information from Queensland includes small numbers of Mary River cod and sleepy cod for some years.

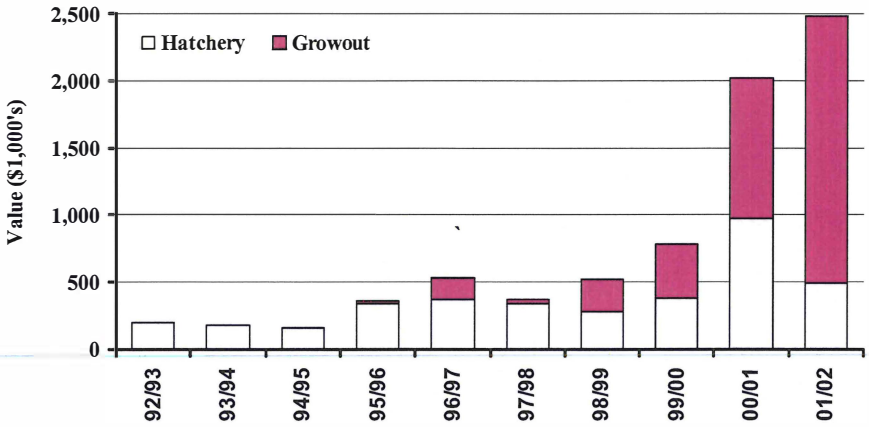

Fig. 2.5. Combined value ($1,000's) of hatchery production of Murray cod fingerlings and growout of table fish from 1992/1993 to 2001/2002

Source: State Government Fisheries aquaculture production statistics.
Note: Information from Queensland includes small numbers of Mary River cod and sleepy cod for some years.

2.5 Conclusions

Murray cod aquaculture is a small but growing industry within Australia. Murray cod responds well to commercial production in RAS. The species is recognised as a premium seafood product as attested by market prices. However, there are still impediments that need to be overcome for the industry to progress and achieve more efficient and higher levels of production in the future. Some areas that require attention include:

- *Seedstock quality and quantity.* Hatchery techniques are well established for production of fingerlings, but availability of seedstock for aquaculture is seasonally limited. Although weaning techniques have been developed some grow-out operators continue to experience problems with fingerlings that have not been properly weaned and/or are in poor condition, many of which do not survive.
- *Culture system design, operation and management.* Continued improvement in the design, set-up and operation of RAS systems for the culture of Murray cod is necessary in order for the table fish sector to continue to expand by culturing a quality, economically viable product with consistent supply to well researched, guaranteed markets.
- *Availability of a cost-effective commercial Murray cod diet.* Although a suitable Murray cod diet has been developed during the present study (see De Silva *et al.* 2004), this has not been taken up by commercial feed manufacturers. For reasons associated with economics and mill performance, the larger feed manufacturers have set minimum milling amounts (>5-10 t) before a specific feed will be produced. Currently the Murray cod aquaculture industry is too small to warrant feed manufacturers developing a line of diets for Murray cod.
- *Fish health.* Water quality requirements for Murray cod are not well known and issues associated with water quality continue to occur in RAS. Similarly, periodic disease problems arise from time to time, especially for inexperienced operators. Fish health management is extremely important and requires greater awareness and education. However, these issues are addressed in later chapters (see Boreham *et al.* 2004; Ingram *et al.* 2004b).
- *Product processing and marketing.* Marketing is an area that needs to be specifically considered. Murray cod is relatively unknown as a table fish to the general public. Therefore, developing existing markets and establishing new markets is required. It is important ensure product quality through all stages of production. Further information on marketing is provided by Larkin *et al.* (2004).

2.6 Acknowledgments

The preparation of this article has involved the willing and generous participation of individuals supplying information from a number of State Fishery Departments, specifically: Mr Dave Glendenning (Aquaculture Extension Officer, NSW Fisheries), Mr Ian Lyall (Aquaculture Manager, NSW Fisheries), Mr Dan Machin (Aquaculture Development Officer, WA Fisheries), Mr Ross Lobegeiger (Supervising Extension Officer, Bribie Island Aquaculture Research Centre, Dept. of Primary Industries, Qld), Ms Tara Ingerson, (Aquaculture Extension Officer, Primary Industries and Resources, SA), Mr Brendan Larkin (PIRVic, Snobs Creek) and numerous Murray cod farmers from the MCANet.

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3 MURRAY COD FINGERLING PRODUCTION AND GROWOUT

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CONTENTS

3.1 Introduction

Prior to the commencement of the present study, research into Murray cod (*Maccullochella peelii peelii*) culture had largely been limited to development of fingerlings production techniques (Rowland 1983; Cadwallader and Gooley 1985; Rowland 1986b; 1988; 1992). Today production of Murray cod fingerlings for stock enhancement programs and for grow-out in aquaculture operations is routine (Ingram *et al.* 2004a).

Since the early 1990's there has been considerable interest in the commercial grow-out of Murray cod for human consumption, which has been driven in part by the premium value and associated ongoing demand for Murray cod in the domestic market (Ingram 2000). In addition, there is considerable interest in Asian markets where there is a perception that Murray cod is very similar in appearance to other premium species in these markets, such as marine cods and groupers (particularly epinephelids and other serranids) and perch-like fishes (eg. Mandarin fish) (Anon 2001; Larkin *et al.* 2004).

The ability to wean juvenile fish onto an artificial diet is deemed an import attribute for successful aquaculture of a species (Webber and Riordan 1976; Shepherd 1988; Pillay 1990). Indeed, the initial feeding and subsequent weaning of larval fish onto artificial diets is considered to be a critical stage of production and a major bottle-neck for industrial expansion of aquaculture (Sorgeloos *et al.* 1995). Some species of freshwater fish which have large larvae, such as salmonids and coregonids, can be readily weaned to artificial diets from the onset of exogenous feeding without the need of a live, natural food (Stevenson 1987; Jones *et al.* 1993). Yet other species require live starter diets, such as rotifers (*Brachionus*) and/or brine shrimp larvae (*Artemia*) before weaning on to an artificial diet (Denson and Smith 1996; Sheikh-Eldin *et al.* 1997; Daniels and Hodson 1999).

Juvenile Murray cod are routinely reared to a fingerling size (0.75-1.5 g, 40-55 mm) in fertilised earthen ponds under ambient conditions, without supplementary feeding (eg. Rowland 1986a; 1986b; 1992). At the onset of exogenous feeding (approximately 10 days of age), post-larvae are usually fed live, newly hatched

Artemia nauplii for several weeks before being stocked into the ponds, but occasionally post-larvae may be stocked into ponds at commencement of feeding rather than using *Artemia* (Fig. 3.1).

Typically Murray cod enter commercial grow-out facilities as pond-reared fingerlings (approximately 45 mm in length and 1 g in weight), and it is at this point of development weaning onto an artificial diet occurs (Fig. 3.1). Preliminary trials conducted at the Marine and Freshwater Resources Institute (PIRVic), Snobs Creek, and by industry, suggest that post-larvae can be weaned onto an artificial diet at, or shortly after, the onset of exogenous feeding, thereby eliminating the need of the pond-rearing phase (Fig. 3.1).

Information on the growth of Murray cod is largely limited to studies on wild fish (Anderson *et al.* 1992; Gooley 1992; Rowland 1998). Few studies have reported on the growth of Murray cod in ponds, cages and tanks, and no studies have provided detailed biological data that is relevant to aquaculture production. Information on the cage culture of Murray cod is limited to a single study by Dakin and Kesteven (1938) in which fish that were held in a cage for 13 weeks only. Barlow and Bock (1981) found that the growth of juveniles stocked into ponds was highly correlated to temperature, and the size range became increasingly pronounced over time, while Forster (1999) found that 1 g fish stocked into farm dams reached 2-3 kg in three years. During trials on diet development for Murray cod, juvenile fish held in 1,000 L tanks grew from 5.3 g to 18.7 g in 12 weeks (1.5%/wk) (at 18-20°C) (Gooley and Anderson 1992). However, preliminary results conducted by both commercial farms and research institutes have suggested that juvenile Murray cod (1-2 g), reared intensively in tanks and fed an artificial diet, can reach a market-size of 600 g within 12 months, with high survival rates (Gooley and Rowland 1993; Ingram *et al.* 1999; O'Sullivan 1999).

The objectives of the present study were to investigate aspects of the aquaculture of Murray cod in particular, the initial weaning and rearing of post-larvae and fingerlings, as well as grow-out under controlled conditions in intensive tank-based recirculating aquaculture systems (RAS) and under ambient conditions in cages. Further, by synthesising collected data this study aimed to provide a synopsis of key production information, growth, survival, fish condition, feeding rates, and food conversion ratios, for Murray cod from post-larvae to market-size fish.

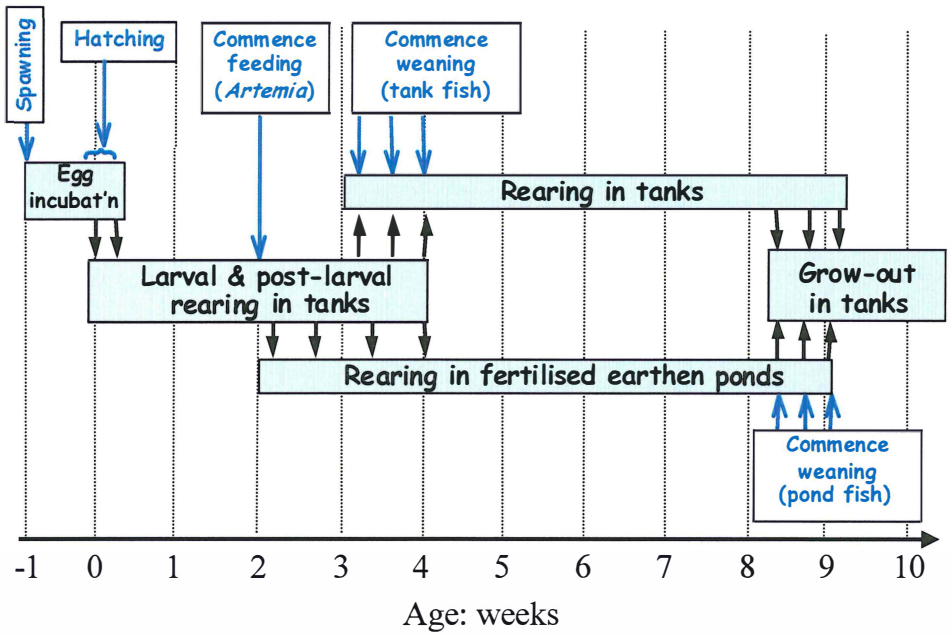

Fig. 3.1. Fingerling production model

3.2 Materials and Methods

A series of replicated and non-replicated trials were undertaken to obtain to key biological information on the initial feeding, weaning and rearing of juvenile Murray cod in tanks, and the growout of Murray cod in tanks and cages. Details of these trials are summarised in Table 3.1.

Trials were conducted at the Marine and Freshwater Resources Institute (PIRVic), Snobs Creek, Victoria, or one of several commercial fish farms. All Murray cod used in trials conducted at PIRVic, Snobs Creek were sourced from the existing Murray cod breeding and stocking program based at PIRVic, Snobs Creek. Descriptions of spawning and fingerling production techniques are given in Rowland (1983, 1986b), Cadwallader and Gooley (1985), Ingram and Larkin (2000), Ingram (2001) and Ingram *et al.* (2004a).

For the purposes of this document, the developmental stages of Murray cod during culture are defined as follows:

Eggs:	Eggs of Murray cod from spawning to hatching
Larvae:	Fish that have hatched but have not commenced feeding, rather relying on yolk-sac reserves.
Post-larvae:	Fish that have commenced exogenous feeding, usually on live, first instar *Artemia* nauplii, but have yet to fully metamorphose, typically less than 25 mm.
Fingerlings:	Fish that are fully metamorphosed, typically 25 - 75 mm. Fish that have been harvested from fry rearing ponds. At this stage fish are fully scaled.
Fry:	Transitional period between post-larvae and fingerlings.
Juveniles:	Young fish that have the appearance of an adult but have yet to reach maturity
Seedstock:	Fish that are stocked into culture systems for grow-out purposes, mainly fry, fingerlings and juveniles.
Weaning:	Transitional period between feeding on live food (*Artemia*, zooplankton etc) or "natural" food (frozen or fresh fish, fish flesh, carp roe etc) and feeding an artificial food (manufactured diets).

3.2.1 Tank culture trials

The majority of tank culture trials were conducted indoors under controlled-environment conditions at PIRVic, Snobs Creek, between December 1999 and May 2002. In general, for these trials, fish were held in 160 L circular fibreglass tanks, which were maintained at a volume of either 50, 100 or 160 L (Fig. 3.2), supplied with a continuous flow of water at temperatures between 20°C and 25°C, at a rate of approximately 1.0–5.0 L/min., and with supplementary aeration (depending on trial requirements). Water supply was from either a continuous flushing system or a recirculation system. In both cases the water was filtered (mechanical and biofiltration), heated to the desired temperature and sterilised (UV light) before being used. Drainage was via a centrally located outlet covered with mesh to prevent escapement (Fig. 3.2).

For each treatment being tested within each trial, 2-4 tanks were used as replicates, and allocation of treatments to each tank was randomised to avoid any inherent bias associated with tank location. Where possible, fish were graded prior to starting each trial to reduce size variation. All tanks were cleaned daily, with uneaten food and faecal material being removed and the sides and floor scrubbed clean.

After the initial allocation of fish to tanks in each trial, random samples of 20-30 individual fish from each tank were anaesthetised and measured. Sedation of fish was achieved using 15-25 mg/L benzocaine (ethyl-p-aminobenzoate), depending on fish size. Accuracy of length and weight measurements was dictated by size of fish. Post-larvae were measured to the nearest 0.001g and 0.1 mm, fingerlings and juveniles to nearest 0.1-0.01g and 0.1 mm and larger fisher (>100 mm) to the nearest 1.0 g and 1.0 mm. Every 2-4 weeks during and at the termination of each trial, a random sample of 20-30 individual fish was measured from each tank. At the beginning and end of each trial the total biomass of each tank was determined by bulk wet weighing. Throughout the trials, mortalities in each tank were recorded daily.

Table 3.1. Summary of tank and cage culture trials conducted on Murray cod during the present study.

Trial description.	Initial mean stocking density	Initial mean fish size	No. fish per replicate	Duration (days)	Treatments	Culture temperature (oC)	Feeding regime
TANK CULTURE TRIALS							
Weaning Post-larvae							
Weaning diet	0.38 kg/m^3 10 fish/L	0.038 g 14.55 mm	1,000	44	Deakin University (DU45%P) Deakin University (DU55%P) Gibsons salmon starter (50%P) Kinta MC Pre-starter	22	Satiation (auto-feeder)
Weaning density Trial 1	0.38-1.15 kg/m^3 10-30 fish/L	0.039 g 14.45 mm	1,000 – 3,000	44	Low density (10 fish/L) Medium density (20 fish/L) High density(30 fish/L)	22	Satiation (auto-feeder)
Weaning density Trial 2	0.35-1.4 kg/m^3 10-40 fish/L	0.035 g 13.81 mm	1,000 – 4,000	21	Low density (10 fish/L) Medium density (20 fish/L) High density(40 fish/L)	24.7	Satiation (six times per day)
Weaning duration	0.7 kg/m^3 20 fish/L	0.035 g 13.67 mm	2,000	21	Nil wean (no weaning period) 1 week weaning period 2 week weaning period	24.7	Satiation (six times per day)
Weaning fingerlings							
Condition at weaning	15 kg/m^3	0.82 g 40.9 mm	920	60	Control Fasted for one week before weaning	22.2	5%/day (belt feeder)
Weaning density	7-30 kg/m^3	0.89 g 41.8 mm	410 – 1,600	60	Low density (7 kg/m^3) Medium density (15 kg/m^3) High density(30 kg/m^3)	22.2	5%/day (belt feeder)
Weaning duration	15 kg/m^3	0.80 g 40.8 mm	920	60	1 week weaning period Nil wean (no weaning period)	22.2	5%/day (belt feeder)
Culture in pond versus culture in tanks	25 fish/m^2 (pond) 10 fish/L (tank)	0.037 g 14.42 mm	7,700-22,370	44	Pond reared Tank reared	22	Various
Weaned as post-larvae versus weaned as fingerlings							
Trial 1	Various	0.0419 g 13.6 mm	5,000 –36,770	315	Weaned as post-larvae, tank reared Pond reared, weaned as fingerlings	18-22	Various
Trial 2	Various	0.05-0.09 g 15.7 –19.9 mm	7,000-21,500	125	Weaned as post-larvae, tank reared Pond reared, weaned as fingerlings	20-22	Various

Table 3.1. Continued

Trial description.	Initial mean stocking density	Initial mean fish size	No. fish per replicate	Duration (days)	Treatments	Culture temperature (oC)	Feeding regime
TANK CULTURE TRIALS							
Effect of water temperature	20 kg/m^3	83 g	24	86	Low temperature (20^{o}C) Medium temperature (22.5^{o}C) High temperature (25^{o}C)	20-26	To satiation (belt feeder)
COMMERCIAL SCALE CULTURE TRIALS							
Commercial feed Trial 1	109 kg/m^3	83 g	2,170	93	Deakin university diet (DU1) Salmon grower diet (45:22)	25	To satiation (belt feeder)
Commercial feed Trial 2	71.7 kg/m^3	81 g	1,410	86	Deakin university diet (DU2) Barramundi diet (45:20)	20	To satiation (belt feeder)
CAGE CULTURE TRIALS							
Cage culture Trial 1	5.5-11 kg/m^3	183 g	60-119	55 (123)	5.5 kg/m^3 11 kg/m^3 (Combined after 55 days)	25 (21)	Satiation (belt feeder)
Cage culture Trial 2	6.6-25 kg/m^3	114 – 343 g	36-220	86	Small (114 g) Low (6.6 kg/m^3) Small (114 g) Medium (16 kg/m^3) Small (114 g) High (25 kg/m^3) Medium (182 g) Low (6.6 kg/m^3) Medium (182 g)High (25 kg/m^3) Large (343 g) Medium (16 kg/m^3)	19.5	Twice per day to set feed rates (0.8-1.0%/day)

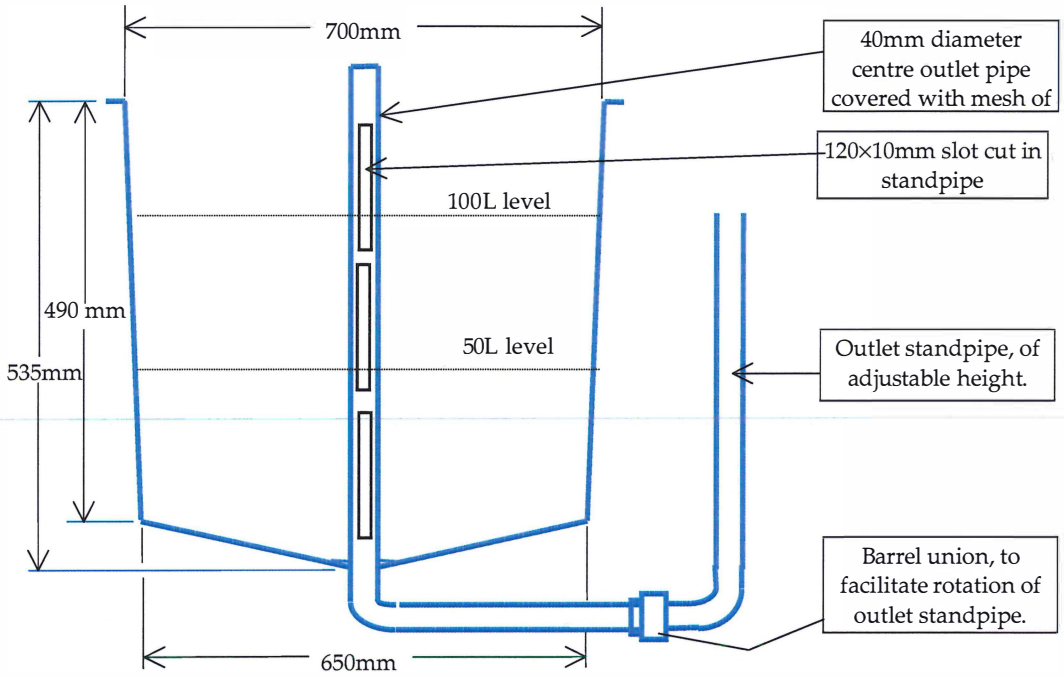

Fig. 3.2 Cross-section of 160 L fibreglass tank used in tank culture experiments at PIRVic, Snobs Creek.

A summary of the proximate composition and energy content of diets and feeds used during trials is presented in Table 3.2. *Artemia* (Aquafauna Bio-marine Inc.) were fed to post-larvae only, according to feed rates presented in Table 3.3. Frozen roe of wild-caught carp (*Cyprinus carpio*) was obtained from K & C Fisheries, Sale, Victoria. Commercial artificial feeds were purchased from feed manufacturers, while Deakin University, Warrnambool, provided experimental artificial feeds.

For artificial feeds, fish were fed to satiation or to set feed rates by hand 3-5 times daily, or by placing known amounts of feed onto "belt-feeders" situated on each tank, which were driven by either a 12 or 24 hr clock mechanism. Set feed rates were dependant on fish size and typically ranged from 1–5%/day (dry weight feed to weight of fish). These rates were adjusted periodically after calculation of change in weight and number of fish in each tank.

Table 3.2. Mean proximate composition and energy content of diets and feeds used in trials during the present study.

Diet	Parameter (mean)				
	Moisture (%)	Protein (%)	Lipids (%)	Ash (%)	Energy (kJ/g)
Artemia (1st instar nauplii)	11	53	38	14	22
Skretting[1] 0.4-0.6 mm salmon starter	9	50	15	10	21
Skretting[1] Salmon crumble (1.5 mm)	9	50	15	10	18
Skretting[1] Salmon grower diet (45:22)[2]	7	45	22	9	23
Kinta MC Pre-starter	3	62	9	11	11
Deakin University (DU45%P)	8	49	14	9	26
Deakin University (DU55%P)	8	60	10	12	24
Deakin University (DU1)[2]	4	49	17	11	22
Deakin University (DU2)[2]	7	49	16	10	21
Skretting[1] Barramundi high energy[2]	8	44	20	12	21
Carp roe	63.2	78.0	17.1	3.8	

1. Formerly Pivot, formerly Gibsons Ltd.
2. Further information on the composition of these diets is provided in De Silva *et al.* (2004).

Table 3.3 ***Artemia*** **feeding rates for Murray cod post-larvae.**

Days feeding	Feeding level	Feeding frequency (feeds per day)	*Artemia* volume (ml per feed) (per 10,000 larvae)	*Artemia* volume (ml per day) (per 10,000 larvae)
1-5	Low	4	6	25
5-10	Medium	6	10 – 15	75
10-25	High	6 – 8	15 - 20	125

3.2.1.1 Weaning post-larvae

Weaning diet

A trial was conducted to determine the effects of different artificial diets on the weaning and subsequent growth and survival of Murray cod post-larvae. Four different diet types were tested (3 replicates per diet). Two diets, Gibsons (Skretting) salmon starter and Kinta MC Pre-starter, were commercially manufactured diets while two, DU45%P (containing 45% protein) and DU55%P (containing 55% protein), were experimental diets specifically formulated for Murray cod by Deakin University, Warrnambool (Table 3.1, Table 3.2). Mean length and mean age of fish at commencement of the trial (1 December 1998) were 14.55 mm and 21 days, respectively. Initial density at commencement of the trial was 10 fish/L.

Prior to commencement of weaning, the fish were initially fed on live 1st instar *Artemia* nauplii according to feeding levels provided in Table 3.3. Fish were offered an artificial diet from day 1, as well as *Artemia*. However, over a 10 day period the amount of *Artemia* provided to each tank was gradually reduced by 10% (by volume) per day. After weaning, fish were fed to satiation using a mechanical feeding system (Mamcarz and Koziowski 1989).

Weaning density

Two separate trials were conducted to determine the effects of stocking density on the weaning, growth and survival of Murray cod post-larvae. During Stocking Density Trial 1, which commenced on 1 December 1998, post-larvae (initial mean length = 14.45 mm. Initial mean age = 21 days) were stocked into replicate tanks (3 replicates per treatment) at densities of either 10 fish/L, 20 fish/L or 30 fish/L (Table 3.1). Fish were weaned according to the schedule described above and were fed Gibsons (Skretting) salmon starter (0.4-0.6 mm) (Table 3.2) to satiation using a mechanical feeding system (Mamcarz and Koziowski 1989). Tank inlet and outlet water quality (for each treatment) was monitored weekly (see section "Water quality analysis").

During Stocking Density Trial 2, which commenced on 31 December 1999, post-larvae (initial mean length = 13.81, initial mean age = 19 days of age) were stocked into tanks at either 10 fish/L, 20 fish/L or 40 fish/L (3 replicates per treatment) (Table 3.1). Fish were weaned according to the schedule described previously and were fed Gibsons (Skretting) salmon starter (0.4-0.6 mm) (Table 3.2) to satiation using belt feeders fitted with 12 hr clock mechanisms during and after weaning. Tank inlet and outlet water quality was monitored weekly (see section "Water quality analysis").

Weaning duration

The effects of duration of the weaning period on growth and survival of Murray cod post-larvae was investigated in a trial (commenced 31 December 1999) in which post-larvae (initial mean age = 19 days, initial mean length =13.67 mm) were weaned for either one week (Fast wean), two weeks (Slow wean), or were placed directly onto an artificial diet without weaning (Nil wean) (3 replicates per treatment) (Table 3.1). Fish were stocked at an initial density of 20 fish/L. Fish weaned for one and two week periods were offered Gibsons (Skretting) salmon starter (0.4-0.6 mm) (Table 3.2) from day 1, as well as *Artemia*. However, over the weaning period the amount of *Artemia* provided to each tank was gradually reduced to nil by the 7th and 14th day for the "Fast wean" and "Slow wean" treatments, respectively. After weaning, fish fed to satiation.

3.2.1.2 Weaning fingerlings

A series of trials were conducted to investigate aspects of the weaning of Murray cod fingerlings (initial mean age = 107 days, initial mean length = 41.3 mm, initial mean weight = 0.84 g) that had previously been reared in a fertilised fry rearing pond. Three trials, which ran concurrently for 60 days, commenced on 28 March 2002. All fish used in the trials were from a single pond (Pond 15) which was harvested on 27 March 2002. In general, fish were weaned from carp roe to Pivot (Skretting) salmon crumble (1.5 mm dia.) (Table

3.2) over one week. During the weaning period, carp roe was fed at a rate of 10%/day for first three days, 5%/day for the following four days, then nil thereafter. Salmon crumble was fed at a rate of 1%/day, 2.5%/day and 5%/day for the first two days, for the following three days, and the remainder of the trial, respectively. Fresh carp roe was placed on a floating raft within each tank daily. Salmon crumble was administered by a belt feeder (fitted with 24 hr clock mechanisms) located on each tank. Uneaten food, which was removed from the tanks daily, dried and weighed in order to correct feed rates.

Condition at weaning
The effect of delaying the commencement of weaning after harvesting from a fry pond on growth and survival of Murray cod fingerlings was investigated. In one treatment, weaning commenced the day after the fingerlings were harvested from a fry pond, while in a second treatment fish were fasted for five days after harvesting before commencing weaning (Table 3.1). Fish were weaned from carp roe to salmon crumble over a one-week period. Fish were stocked at an initial density of 15 kg/m^3.

Weaning density
The effects of stocking density during weaning on growth and survival of Murray cod fingerlings was investigated in a trial in which fish were stocked into replicate tanks at densities of either 7 kg/m^3, 15 kg/m^3 or 30 kg/m^3 (Table 3.1). Fish were weaned from carp roe to salmon crumble over a one-week period as described above.

Weaning duration
In a non-replicated trial, the effects of duration of the weaning period on growth and survival of Murray cod fingerlings was investigated. One group of fish were weaned from carp roe to the salmon crumble over a 1-week period while a second group were placed directly onto the salmon crumble without being offered carp roe (Table 3.1). Fish were stocked at an initial density of 15 kg/m^3.

3.2.1.3 Culture in ponds versus culture in tanks

Historical pond culture data
At PIRVic, Snobs Creek, five earthen ponds (0.09-0.4 ha) are used to rear juvenile Murray cod. Typically these ponds are stocked about 2 weeks after filling and harvested 5-7 weeks following stocking. At stocking fish are about 15 mm in length (0.09 g) and 30 days of age. Ponds are typically stocked (up to 25 fish/m^2) when zooplankton densities exceed 500 ind./L (with a high abundance of cladocerans and copepods) (Ingram 2001). Operation and management of these ponds are described in Arumugam (1986), Rowland (1986a), Rowland (1986c), and Ingram (2001). A total of 14 seasons of data, representing 59 pond stockings of Murray cod were summarised to provide historical information on growth (SGR) and survival of juvenile Murray cod during pond culture. These data were compared with data from fish weaned as post-larvae and reared in tanks under controlled conditions collected during the present study.

Pond culture verus tank culture
A non-replicated trial was conducted to compare the growth and survival of pond-reared fish against tank-reared fish. On 1 December 1998, post-larvae from a single spawning were either stocked into a 0.09 ha pond (22,370 fish) at a rate of 25 fish/m^2, or stocked into three 160 L tanks (3,000 fish per tank) at 10 fish/L (Table 3.1). Initial mean length and mean age at stocking were 14.42 mm and 30 days, respectively. Tank-reared fish were weaned from live *Artemia* on to Gibsons (Skretting) 0.4-0.6 mm salmon starter (Table 3.2) over a two week weaning period (see Section "Weaning larvae"), and were subsequently fed to satiation on that diet for the duration of the trial. Pond-reared fish were reared under ambient conditions on a diet of live, naturally occurring zooplankton and macroinvertebrates (Ingram 2001).

3.2.1.4 Weaning as post-larvae versus weaning as fingerlings

Two separate non-replicated trials were conducted to compare the growth and survival of juvenile Murray cod that were either grown in a pond and weaned as fingerlings after harvest, or weaned as post-larvae and reared in tanks (Fig. 3.1). The treatments in each trial were:

- *Weaned as post-larvae and tank reared*: Post-larvae were stocked into tanks and weaned from live *Artemia* onto Gibsons (Skretting) 0.4-0.6 mm salmon starter (Table 3.2) (as described in Section "Weaning post-larvae").

- *Pond-reared and weaned as fingerlings*: Post-larvae were stocked into a fertilised earthen pond (see Section

"Historical pond culture data"). At harvest fingerlings were transferred to tanks and weaned onto salmon crumble (1.5 mm dia) (Table 3.2) (see Section "Weaning fingerlings").

In a preliminary trial (Trial 1), which commenced on 9 January 1998, post-larvae from mixed Murray cod spawnings were stocked into a fertilised 0.24 ha pond (Pond 13), or transferred to three 160 L tanks and weaned onto an artificial diet. At harvest of the pond (9 Feb 1998), fingerlings were transferred to tanks and weaned onto an artificial diet. On the 4 August 1998 fish that were weaned as post-larvae were graded into two size classes. The length and weights of fish in each treatment were measured every 1-4 weeks for a period of 45 weeks.

In a second trial (Trial 2), commencing 20 November 2001, post-larvae from mixed Murray cod spawnings were transferred to two 160 L tanks and weaned onto an artificial diet, or were stocked into a fertilised 0.09 ha pond (Pond 15). At harvest of the pond, fingerlings were transferred to a 1,000 L fibreglass tank and weaned onto an artificial diet. The length and weight of a sample of fish in each treatment were determined every 1-4 weeks for a period of 22 weeks. Survival was monitored daily.

3.2.2 Effect of water temperature on growth

The effects of water temperature on growth and feeding of juvenile Murray cod (initial weight 83 g) were investigated in a replicated trial (three tanks per treatment) in which fish were reared in three different water temperature regimes over a period of 86 days (commencing 17 August 2000). The water temperature regimes and sources of water used for each treatment were:

Low temperature (20°C):	Snobs Creek ambient (flow through)
Medium temperature (22.5°C):	Recirculation system
High temperature (25°C):	Recirculation system.

Water temperature in each treatment was monitored with a data logger (Datataker 100), which recorded the daily maximum, minimum and mean temperature from measurements taken every 15 min. Other water quality parameters, including dissolved oxygen (DO), pH, ammonia, nitrite, phosphate and alkalinity, were monitored weekly (see section "Water quality analysis"). Fish were fed a commercial extruded salmon diet (45:22) (slow sinking) (Table 3.2) to satiation using belt feeders located on each tank. The amount of feed given each day was adjusted according to observed feeding levels.

3.2.3 Commercial scale culture trials

Two separate feed trials were undertaken on two fish farms at which Murray cod were being grown intensively in recirculating aquaculture systems (RAS) under commercial conditions. These trials were:

3.2.3.1 Feed Trial 1

The first trial, which ran for 93 days, commencing 7 August 2000, was undertaken at Australian Aquaculture Products Pty Ltd (AAP), Euroa, Victoria (Plate 1e & Plate 2c). Fish (initial weight 83 g) were reared in 1,600 l circular plastic tanks, which were part of a Hesy intensive RAS (O'Sullivan 1999). Fish were fed either a salmon grower diet (45:22) or an experimental diet, Deakin University (DU1) (two replicates per treatment) to satiation (Table 3.2). A more detailed description of the diets and nutritional aspects of this trial are provided in De Silva *et al.* (2004). Diets were fed into each tank over a 24 h period via automated belt feeders. On Day 31 of the trial, each tank of fish was split into two tanks to reduce densities (four replicates per treatment). During the trial, water temperature was maintained at approximately 25°C and water temperature, DO (as % saturation) and pH were measured every two hours using a TPS datalogger (see section "Water quality analysis"). Additional water quality parameters (ammonia, nitrite and nitrate) were monitored daily by AAP staff.

3.2.3.2 Feed Trial 2

The second trial, which ran for 86 days, commencing 12 September 2001, was undertaken at the Alexandra Fish Farm (AFF), Alexandra, Victoria. Fish (initial weight 81 g) were reared in 1,600 l circular plastic tanks within a Hesy intensive recirculating aquaculture system, which is described in detail by Boreham *et al.* (2004). Fish were fed either a high-energy barramundi diet (45:20) or an experimental diet, Deakin University (DU2) (2 replicates per treatment) to satiation. Nutritional aspects of this trial are discussed in De Silva *et al.* (2004). Diets were fed into each tank over a 24 h period via automated belt feeders. During the

trial, water temperature was maintained at approximately 20°C and water temperature, DO (as % saturation) and pH were measured every two hours using a TPS datalogger (see section "Water quality analysis"). Additional water quality parameters (ammonia, nitrite and nitrate) were monitored weekly.

3.2.3.3 Growth of 1999 cohort

Over a 300-day period, the growth of a single cohort of juvenile Murray cod seedstock was monitored at the AAP. Juvenile Murray cod were obtained from a commercial Murray cod fingerling producer. Fish that were 29.9 mm in length and 0.8 g in weight were stocked into the system on 13th January 2000. Using a length-for-age relationship determined for juvenile Murray cod (Ingram 2001), the mean age of these fish was estimated to be 65 days. Every 3-4 weeks, for 43 weeks, lengths and weights of fish were monitored and SGR's determined. Survival rates were not monitored as fish were regularly graded, which meant that some fish were mixed into other cohorts, making it difficult to follow specific groups of fish. Fish were initially placed into 100 L tanks in a quarantine system. When sufficient biomass was available these fish were moved into 1,600 L tanks within the main production system, and were subsequently transferred to 15,000 L tanks for grow-out. Water temperature and DO (% saturation) in the system were maintained at 23-26°C and >90%, respectively. Water flow rates were 4.5-6.5 m^3/hr. Fish were fed to set feed rates (administered over 24 hours by belt feeder) using commercial diets specifically formulated for either salmon or barramundi, depending on size of fish. Temperature, pH and DO (as % saturation) were monitored with a TPS data logger (see section "Water quality analysis"). These data were supplemented with water quality date collected daily by AAP staff.

3.2.4 Cage culture trials

In order to determine the performance of Murray cod reared in cages in static earthen ponds, two non-replicated pilot-scale cage culture trials were conducted during the present study. These were:

3.2.4.1 Cage culture Trial 1

The first trial, which ran for 123 days, was undertaken at a commercial silver perch (*Bidyanus bidyanus*) farm near Torrumbarry, Vic. On 11th January 2001, 179 Murray cod (initial mean weight 183 g), which had been reared in tanks at PIRVic, Snobs Creek, were transferred to two floating 2 m^3 cages situated in a 0.4 ha earthen pond containing silver perch at Torrumbarry, Victoria (Plate 2e). Cages were constructed from 12 mm square polyurethane mesh attached to a 90 mm PVC pipe flotation collar. One cage was stocked with 60 fish (5.5 kg/m^3) and the second with 119 fish (11 fish/m^3). Fish were fed a commercial extruded salmon diet (45:22) (slow sinking) (Table 3.2) to apparent satiation using belt feeders situated on each cage. After 55 days, the fish were combined into one cage and reared for another 68 days.

3.2.4.2 Cage culture Trial 2

The second trial was, which ran for 86 days from 25 February 2002 to 22 May 2002, was conducted at PIRVic, Snobs Creek (Plate 2f) to investigate growth, survival and food conversion efficiency of three size classes (small = 114 g, medium = 182 g, large = 343 g) of Murray cod stocked into 1 m^3 cages at three different densities (low = 6.6 kg/m^3, medium = 16 kg/m^3, high = 25 kg/m^3). A total of 6 cages, attached to a floating pontoon in a 0.24 ha pond, were stocked with fish (one replicate per treatment) (Plate 2f). Small-sized fish were stocked at small, medium and high densities, medium-sized fish at low and high densities and large-sized fish at the medium density only (Table 3.1). Fish were fed by hand twice daily with a commercial extruded barramundi diet (45:20) (slow sinking) (Table 3.2) to set feed rates (0.8-1.0%/day).

In both trials, fish were exposed to ambient conditions. Netting was placed over the tops of the cages to prevent escape and bird predation. Supplementary aeration was provided in each pond by a mechanical paddlewheel, which was positioned near the cages.

3.2.5 Water quality analysis

Dissolved oxygen (DO), temperature and pH were measured *in situ* in each tank using a portable water quality meters, 1-3 times each week. In some trials, temperature, DO (as mg/L or % saturation) and pH were measured *in situ* every 2 hours with a TPS 90 series data logger. Total Ammonia Nitrogen (TAN) (Nessler Method), nitrite-nitrogen (NO_2-N) (Diazotization method), nitrate-nitrogen (NO_3-N) (Cadmium reduction method), total Phosphorus (total P) as phosphate (Acid Persulphate Digestion Method), suspended solids and turbidity were measured using a Hach 4000 spectrophotometer, 1-3 times weekly. Total alkalinity was determined by sulphuric acid titration. Biochemical oxygen demand (BOD_5) was determined using "WTW

Oxitops " incubated at 20°C for five days. Unionised ammonia (UIA) was determined from formulae provided by Emerson *et al.* (1975).

3.2.6 Data analysis

Specific growth rates (SGR's), which were expressed as the percentage increase in body weight per day (%/day) were determined by using the formula:

$$Specific\ \ Growth\ \ Rate\ \ (SGR) = \frac{(\ln Wt_2 - \ln Wt_1)}{(t_2 - t_1)} \times 100\,\%$$

where: t = time in days.
$\ln W_{t2}$ = natural logarithm of the average weight at time *t2*.
$\ln W_{t1}$ = natural logarithm of the average weight at time *t1*.

SGR's were used to compare growth rates of fish within each trial only. Due to variations in SGR's associated with age/size of fish, water temperatures and other factors, no comparisons of SGR's were drawn between specific trials.

Fish condition was determined for individual fish by applying the formula:

$$Condition = \frac{Weight\ (g)}{total\ length\ (cm)^3} \times 100$$

Food Conversion Ratio (FCR) was determined by the formula:

$$Food\ \ Conversion\ ratio\ \ (FCR) = \frac{Food\ \ consumed\ \ (g\ dry\ Wt)\ \ between\ \ t_1\ \ and\ \ t_2}{Increase\ \ in\ \ fish\ \ biomass\ \ (g\ wet\ Wt)\ \ between\ \ t_1\ \ and\ \ t_2}$$

where: t_1 = Initial time.
t_2 = Final time.
Wt_2 = Final fish weight (g) (at time t_2).
Wt_1 = Initial fish weight (g) (at time t_1).

To test for a significant difference between treatments (where $P<0.05$), analysis of fish growth (treating time as a covariant), SGR, final weight, length, and condition, survival, feed rate and FCR for each trial in which treatments were replicated were undertaken using the SAS General Linear Models Procedure and Tukey's Multiple Range Test (SAS Institute Inc.). Prior to analysis, data sets that were identified as heterogeneous by using Cochran's Test for homogeneity were log transformed.

3.3 Results

3.3.1 Tank culture trials

3.3.1.1 Weaning post-larvae

Weaning diet

After 44 days, SGR (P=0.0291), final weight (P=0.01) and final condition (P=0.02) of Murray cod post-larvae were significantly different from each other after being weaned onto different artificial diets (Table 3.4). The final weight of fish that were fed the Gibsons salmon starter diet was significantly greater than for all other diets whereas the final weight of fish fed the Deakin University 45%P diet was significantly less than for all other diets (Fig. 3.3). However, final length and survival were not significantly affected by diet type. An initial drop in survival occurred between 10 and 16 days after commencement of weaning, which was due to infestation by white spot (*Ichthyophthirius multifiliis*). However, fish were treated with salt bathes and mortalities thereafter.

Table 3.4. Initial and final weight, length, condition, specific growth rate (SGR) and survival rate of Murray cod post-larvae during tank culture trials (values = mean ± s.e.).

Parameter	Treatment*			
Weaning diet	*Deakin University 45%P*	*Deakin University 55%P*	*Gibsons salmon starter*	*Kinta Murray cod pre-starter*
Mean Length (mm)				
Initial	14.7 ± 0.1	14.6 ± 0.1	14.4 ± 0.1	14.6 ± 0.1
Final	21.0 ± 0.1	21.8 ± 0.3	22.8 ± 0.4	21.7 ± 0.2
Mean weight (g)				
Initial	0.039 ± 0.001	0.038 ± 0.001	0.038 ± 0.001	0.038 ± 0.001
Final	0.10 ± 0.002^{c}	0.121 ± 0.005^{b}	0.158 ± 0.008^{a}	0.119 ± 0.003^{b}
Condition				
Initial	1.24 ± 0.02	1.21 ± 0.02	1.29 ± 0.03	1.25 ± 0.02
Final	1.08 ± 0.02^{b}	1.15 ± 0.03^{ab}	1.33 ± 0.03^{a}	1.17 ± 0.03^{ab}
Survival rate (%)	49 ± 3	50 ± 26	33 ± 17	37 ± 193
SGR (%/day)	2.25 ± 0.04^{b}	2.70 ± 0.50^{ab}	3.65 ± 0.14^{a}	2.69 ± 0.04^{ab}
Weaning density 1	*Low (10 fish/L)*	*Medium (20 fish/L)*	*High (30 fish/L)*	
Mean Length (mm)				
Initial	14.4 ± 0.1	14.5 ± 0.1	14.4 ± 0.1	
Final	22.8 ± 0.4^{a}	23.3 ± 0.3^{a}	26.0 ± 0.4^{b}	
Mean weight (g)				
Initial	0.038 ± 0.001	0.040 ± 0.001	0.038 ± 0.001	
Final	0.158 ± 0.008^{a}	0.179 ± 0.005^{a}	0.245 ± 0.008^{b}	
Condition				
Initial	1.29 ± 0.03	1.30 ± 0.03	1.26 ± 0.02	
Final	1.33 ± 0.03	1.42 ± 0.03	1.39 ± 0.03	
Survival rate (%)	33 ± 17	63 ± 8	68 ± 3	
SGR (%/day)	3.65 ± 0.14	3.55 ± 0.33	4.42 ± 0.28	
Weaning density 2	*Low (10 fish/L)*	*Medium (20 fish/L)*	*High (40 fish/L)*	
Mean Length (mm)				
Initial	13.9 ± 0.1	13.7 ± 0.1	13.8 ± 0.1	
Final	19.0 ± 0.2^{a}	17.0 ± 0.2^{b}	17.5 ± 0.2^{b}	
Survival rate (%)	61 ± 4	36 ± 10	32 ± 7	
Weaning duration	*No wean (0 days)*	*Fast wean (7 days)*	*Slow wean (14 days)*	
Mean Length (mm)				
Initial	13.6 ± 0.1	13.7 ± 0.1	13.7 ± 0.1	
Final	17.9 ± 0.2^{a}	17.4 ± 0.2^{ab}	17.0 ± 0.2^{b}	
Survival rate (%)	37 ± 15	46 ± 6	36 ± 10	

* Treatments with the same letter (superscript) are not significantly different from each other (Tukey's Studentised Range Test)

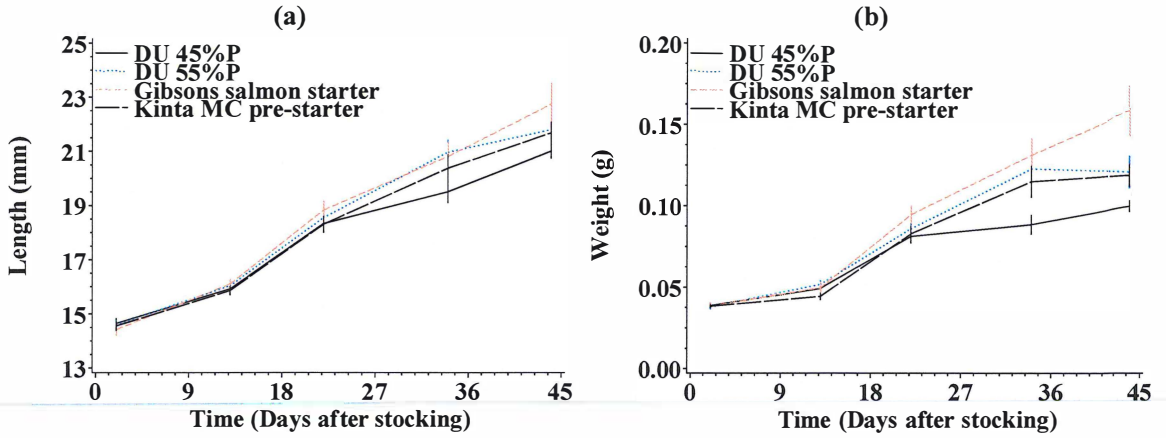

Fig. 3.3. Change in (a) length (mean ± s.e.) and (b) weight (mean ± s.e.) of Murray cod post-larvae weaned onto four different artificial diets.

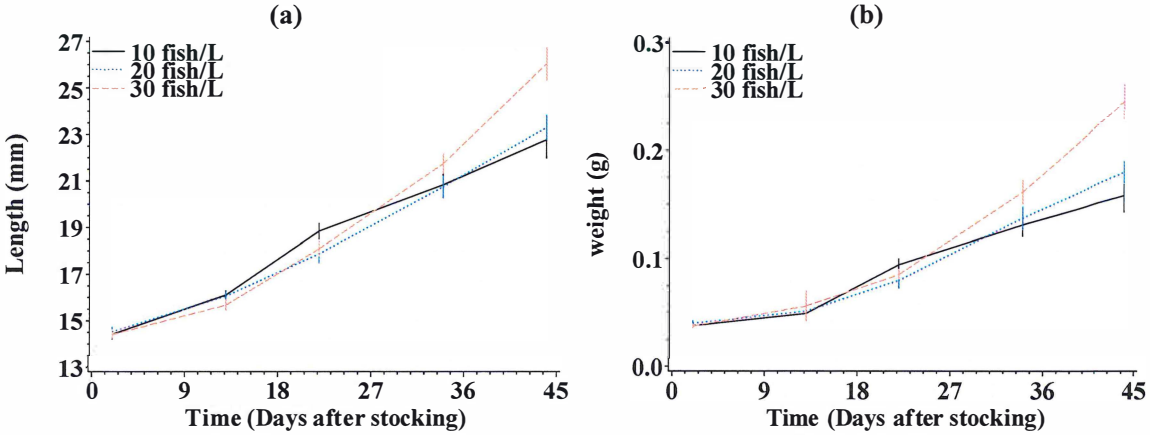

Fig. 3.4. Change in (a) length (mean ± s.e.) and (b) weight (mean ± s.e.) of Murray cod post-larvae .reared at three different densities.

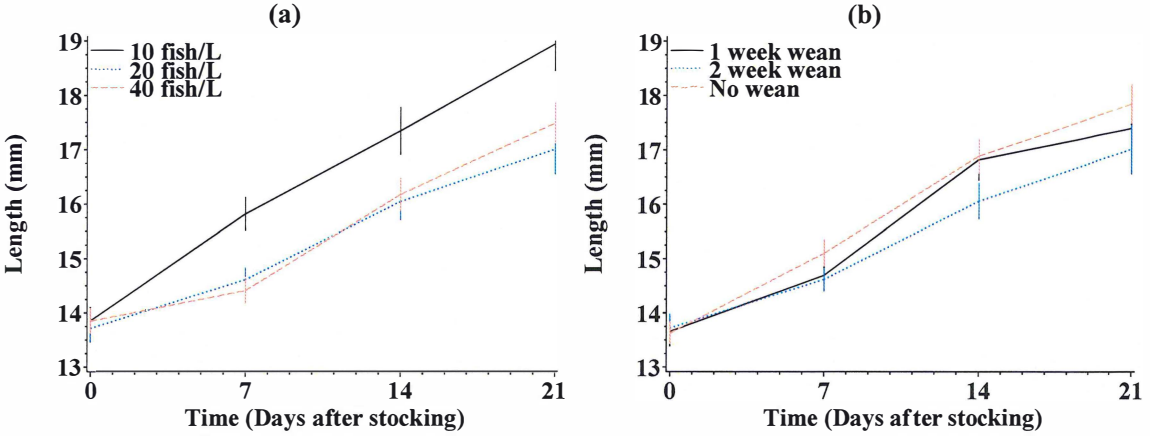

Fig. 3.5. Change in length (mean ± s.e.) of Murray cod post-larvae (a) stocked at three different densities and (b) weaned over three different periods of time.

Weaning density
During stocking density Trial 1, both change in weight and length over time were significantly affected by stocking density (weight: P=0.0338. Length: P=0.0287) (Fig. 3.4). Both final weight and length of fish, that were initially stocked at 30 fish/L, were significantly greater (P<0.001) than for fish stocked at lower densities (Table 3.4). Yet, SGR and final condition of fish were not significantly affected by stocking density. Survival rates were highly variable across the replicates (Table 3.4). Despite the low mean survival rate in the low density treatment (10 fish/L), survival was not significantly affected by stocking density. However, as with the weaning diet trial, fish were infested with white spot in the early weeks of the trial. TAN and total phosphorus concentrations in inlet water samples were generally lower than in outlet water samples, whereas pH tended to be greater in inlet water samples (Table 3.5). As stocking density increased, concentrations of TAN and total phosphorus increased and pH declined in outlet water samples (Table 3.5).

During stocking density Trial 2, Murray cod reared in tanks at an initial density of 10 fish/L grow significantly faster than fish reared in tanks at higher densities (20 fish/L and 40 fish/L) (P=0.0288) (Table 3.4, Fig. 3.5a). Survival of Murray cod post-larvae was highly variable and not significantly affected by stocking density (P=0.0686).

Weaning duration
Change in length over time was significantly affected by duration of the weaning period (P=0.0263) (Table 3.4). Murray cod that were weaned over two weeks grow more slowly than fish placed directly onto an artificial without a weaning period (Nil wean) (Fig. 3.5b). Although survival of Murray cod was not significantly affected by weaning duration (P=0.7942), the rate of mortality in the nil wean treatment was substantially greater than other treatments during the early weeks of the trial.

Water quality data recorded during the trial are summarised in Table 3.5. Both TAN and total phosphorus concentrations were significantly greater (P<0.05) in the outlet water samples than in inlet water samples.

Table 3.5. Water quality variables (mean ± s.e.) measured during tank culture trials investigating the weaning of Murray cod post-larvae.

Parameter	Inlet	Treatment/outlet*		
Weaning density Trial 1	*Inlet*	*10 fish/L*	*20 fish/L*	*30 fish/L*
Temperature (oC)	19.4 (0.5)	19.5 (0.4)	19.4 (0.5)	19.6 (0.5)
DO (mg/L)	8.14 (0.19)	7.61 (0.24)	7.47 (0.33)	7.43 (0.33)
PH	6.74 (0.02)^{a}	6.70 (0.01)^{ab}	6.64 (0.02)^{c}	6.65 (0.01)^{bc}
TAN (mg/L)	0.10 (0.01)^{a}	0.13 (0.01)^{ab}	0.16 (0.01)^{b}	0.16 (0.02)^{b}
Total phosphorus (mg/L)	0.13 (0.01)^{a}	0.20 (0.01)^{b}	0.25 (0.02)^{b}	0.20 (0.02)^{b}
Weaning duration	*Inlet*	*Outlet*		
Temperature (oC)	24.7 (0.22)			
DO (mg/L)	7.5 (0.12)			
PH	6.58 (0.11)	6.62 (0.07)		
TAN (mg/L)	0.19 (0.04)^{a}	1.08 (0.43)^{b}		
Total phosphorus (mg/L)	0.36 (0.06)^{a}	1.17 (0.39)^{b}		

* Treatments with the same letter (superscript) are not significantly different from each other (Tukey's Studentised Range Test).

3.3.1.2 Weaning fingerlings

Condition at weaning
Change in both length and weight over time was significantly affected by delaying the commencement of weaning after harvesting from a fry pond (P=0.002) (Fig. 3.6). Murray cod starved for five days prior to commencing weaning onto an artificial diet had a lower SGR, condition, body weight and length at the end of the trial (Table 3.6). However, the survival rate of starved fish was higher than for fish weaned immediately after harvesting, though this trend was not significant.

Weaning density
Murray cod fingerling stocked into tanks at higher densities during weaning had slower growth rates than those stocked at lower densities (Fig. 3.7). By the end of the trial, fingerlings stocked at 30 kg/m^3 had a significantly lower mean weight (2.75 g) (P=0.0015) and mean length (60.8 mm) (P=0.001) than fish stocked at

7 kg/m^3 (mean weight = 3.8 g, mean length = 68 mm) (Table 3.6). However, the survival rate was highest in tanks stocked at the highest density (30 kg/m^3 = 71%), though not significantly so (Table 3.6).

Weaning duration
In a non-replicated trial, fingerling Murray cod that were offered salmon crumble without a weaning phase incorporating carp roe, had a slightly higher SGR (2.64%/day) and survival rate (63%) than fish that were weaned using carp roe (SGR = 2.21%/day; survival = 50%) (Fig. 3.8, Table 3.6).

Adjusted feed rates during fingerling weaning trials ranged from 3.76%/day (stocking density treatment 7 kg/m^3) to 4.2%/day (fish weaned over 1 week, stocked at 15 kg/m^3). Survival rates during fingerling weaning trials ranged from 50-71%. In all tanks, there were signs of aggression, tail biting and cannibalism, which may have contributed to the low survival rates observed during these trials.

3.3.1.3 Culture in ponds versus growth in tanks

Historical pond culture data
Between 1988 and 2002, a total of 59 pond stockings of juvenile Murray cod were undertaken at PIRVic, Snobs Creek. Ponds were stocked as early as late November and as late as early March. At stocking fish ranged from 17 to 63 (mean 29) days of age. Fish were held in ponds for up to 87 days (mean 40 days) before being harvested.

The length-weight, length-age and weight-age relationships (determined by regression) (see Ingram 2001) for pond-reared juvenile Murray cod were:

Log weight = -10.992 (± 0.040 s.e.) + 2.888 (± 0.011 s.e.) x Log length (adj. $R^2 = 0.99$)
Log length = -0.145 (± 0.078 s.e.) + 0.925 (± 0.019 s.e.) x Log age (adj. $R^2 = 0.80$)
Log weight = -11.372 (± 0.222 s.e.) + 2.729 (± 0.055 s.e.) x Log age (adj. $R^2 = 0.82$)

At stocking, fish were 11.0 to 20.7 mm (mean 14.7 mm) in length and 0.034 to 0.1 g (mean 0.036 g) in weight. Stocking densities ranged from 2.7 to 32.2 fish/m^2 (mean 16.5 fish/m^2). At harvest, fish were 33.8 to 56.9 mm (mean 44.0 mm) in length and 0.44 to 2.04 g (mean 1.04 g) in weight. Growth of fish ranged from 0.39 to 1.07 mm/day (mean 0.71 mm/day), and SGR's ranged from 4.37 to 11.64 %/day (mean 7.78 %/day). SGR was negatively correlated with size at stocking. Condition at harvest ranged from 0.71 to 1.46 (mean 1.15). Both SGR and condition were highest in the weeks immediately following stocking, but tended to decline in latter weeks. Survival rates ranged from 30.4 to 96.5% (mean 72.6%). Water quality measured in ponds during the rearing of Murray cod is presented in Table 3.7. Diet by dry weight of Murray cod during pond culture was composed of chironomid larvae (51%), calanoids (12%), *Moina* (11%), cyclopoids (5%) and other invertebrates (21%).

Pond culture versus tank culture
Growth in length and weight of Murray cod reared in fertilised earthen fry rearing ponds was substantially greater than for fish weaned onto an artificial diet and reared in tanks (Fig. 3.9). SGR of fish reared in ponds was greater (8.36%/day) than for fish reared in tanks (4.42%/day) (Table 3.8). However, the final condition of tank-reared fish (mean 1.39) was considerably greater than pond-reared fish (mean 1.15) (Table 3.8). Survival rates were 95% and 68% for pond-reared and tank-reared fish, respectively.

Weaning as post-larvae versus weaning as fingerlings
Two trials were undertaken investigate the growth of fish either weaned as post-larvae and reared in tanks, or reared in ponds then weaned as fingerlings. In both trials, growth rates were greatest in fish reared in ponds, and pond-reared fish were generally larger than fish of a similar age that had been weaned as post-larvae and reared in tanks on an artificial diet (Fig. 3.10, Fig. 3.11). However, following harvest the growth of fingerlings declined during the weaning period, which was most evident in Trial 2 (Fig. 3.11). During Trial 2, fish weaned as post-larvae and tank-reared were considerably smaller than fingerlings at the time of harvest from the pond (Fig. 3.11). However, in the month following harvest and weaning the growth of the pond-reared fingerlings declined noticeably whereas the growth of fish weaned as post-larvae and tank-reared was maintained. By the end of the trial, fish weaned as post-larvae were greater in weight and length, and had a higher SGR than fish weaned as fingerlings (Table 3.8). In contrast, during Trial 1, despite a slowing in growth of pond-reared fish following harvest and weaning, at the end of the trial fish weaned as fingerlings were larger than fish weaned as post-larvae (Fig. 3.10, Table 3.8).

Table 3.6. Initial and final weight, length, condition, specific growth rate (SGR), survival rate, FCR and feed rate of Murray cod fingerlings during weaning trials (values = mean ± s.e.).

Parameter		Treatment*		
Condition at weaning		*Starved*	*Control*	
Mean Length (mm)	- Initial	40.4 ± 0.6	41.5 ± 0.6	
	- Final	59.2 ± 1.1[a]	63.8 ± 1.4[b]	
Mean weight (g)	- Initial	0.77 ± 0.04	0.86 ± 0.04	
	- Final	2.51 ± 0.14[a]	3.26 ± 0.21[b]	
Condition	- Initial	1.14 ± 0.01	1.17 ± 0.02	
	- Final	1.17 ± 0.01	1.19 ± 0.02	
Survival rate (%)		60 ± 5	50 ± 8	
SGR (%/day)		1.96 ± 0.16	2.21 ± 0.10	
Feed rate (%)		3.92 ± 0.10	4.20 ± 0.14	
FCR		3.63 ± 1.18	4.85 ± 1.9	
Weaning density		*Low (7 kg/m³)*	*Medium (15 kg/m³)*	*High (30 kg/m³)*
Mean Length (mm)	- Initial	41.7 ± 0.5	41.5 ± 0.6	42.4 ± 0.6
	- Final	67.9 ± 1.3[a]	63.8 ± 1.4[ab]	60.8 ± 1.2[b]
Mean weight (g)	- Initial	0.86 ± 0.04	0.86 ± 0.04	0.93 ± 0.04
	- Final	3.81 ± 0.23[a]	3.26 ± 0.21[ab]	2.75 ± 0.17[b]
Condition	- Initial	1.16 ± 0.02	1.17 ± 0.02	1.19 ± 0.02
	- Final	1.17 ± 0.01	1.19 ± 0.02	1.16 ± 0.01
Survival rate (%)		64 ± 2	50 ± 8	71 ± 3
SGR (%/day)		2.48 ± 0.21	2.21 ± 0.10	1.78 ± 0.28
Feed rate (%)		3.76 ± 0.13	4.20 ± 0.14	4.01 ± 0.26
FCR		2.32 ± 0.42	4.53 ± 1.0	3.39 ± 0.76
Weaning duration		*No wean*	*7 day wean*	
Mean Length (mm)	- Initial	39.7 ± 0.5	41.6 ± 0.6	
	- Final	64.8 ± 1.3	63.8 ± 1.4	
Mean weight (g)	- Initial	0.71 ± 0.03	0.86 ± 0.04	
	- Final	3.47 ± 0.22	3.26 ± 0.21	
Condition	- Initial	1.12 ± 0.01	1.16 ± 0.02	
	- Final	1.24 ± 0.01	1.19 ± 0.02	
Survival rate (%)		63	50 ± 8	
SGR (%/day)		2.64	2.21 ± 0.10	
Feed rate (%)		4.05	4.20 ± 0.14	
FCR		2.51	4.85 ± 1.9	

* Treatments with the same letter (superscript) are not significantly different from each other (Tukey's Studentised Range Test)

Table 3.7. Summary of water chemistry parameters measured in fry rearing ponds at PIRVic Snobs Creek between 1998 and 2002.

Parameter	Mean	Range	Standard error
Temperature (°C)	22.5	14 - 34.0	0.05
DO (mg/L)	8.08	1.18 – 18.20	0.05
pH	7.91	5.60 – 10.38	0.02
TAN (mg/L)	0.40	0.01 – 1.93	0.01
NO_3-N (mg/L)	0.23	0 – 1.50	0.01
NO_2-N (mg/L)	0.003	0 – 0.019	0.0002
UIA (mg/L)	0.02	0 – 0.58	0.003
Orthophosphate- (mg/L)	0.74	0-2.80	0.03
Total alkalinity (mg/L)	42.9	1.2 – 194.6	0.79
Secchi disk visibility (m)	0.63	0.12 - >1.3	0.01

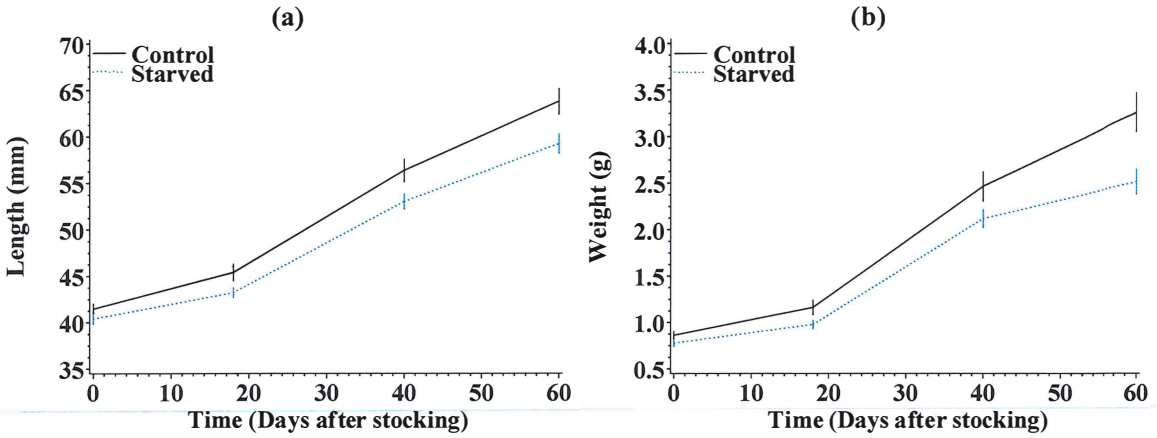

Fig. 3.6. Effect of a delay in weaning on (a) length (mean ± s.e.) and (b) weight (mean ± s.e.) of Murray cod fingerlings during and after weaning

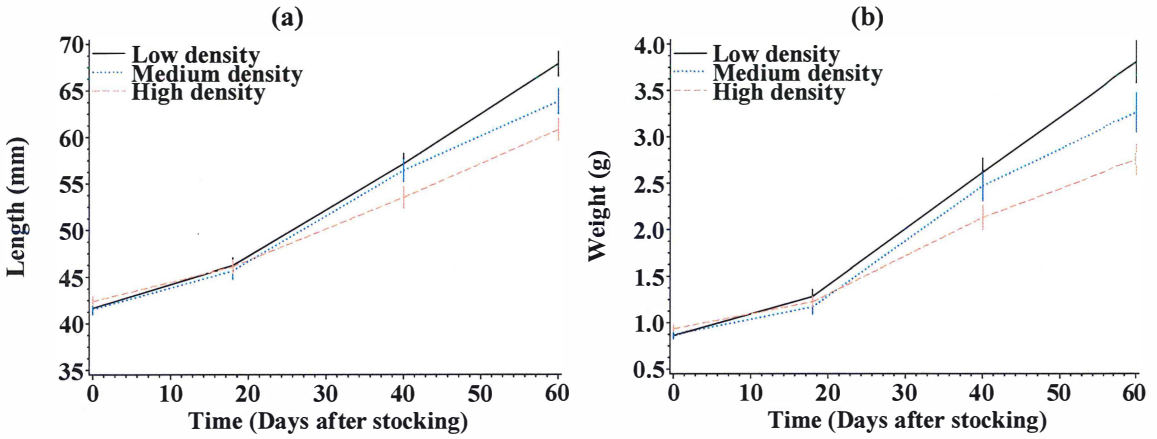

Fig. 3.7. Effect of a stocking density on (a) length (mean ± s.e.) and (b) weight (mean ± s.e.), of Murray cod fingerlings during and after weaning.

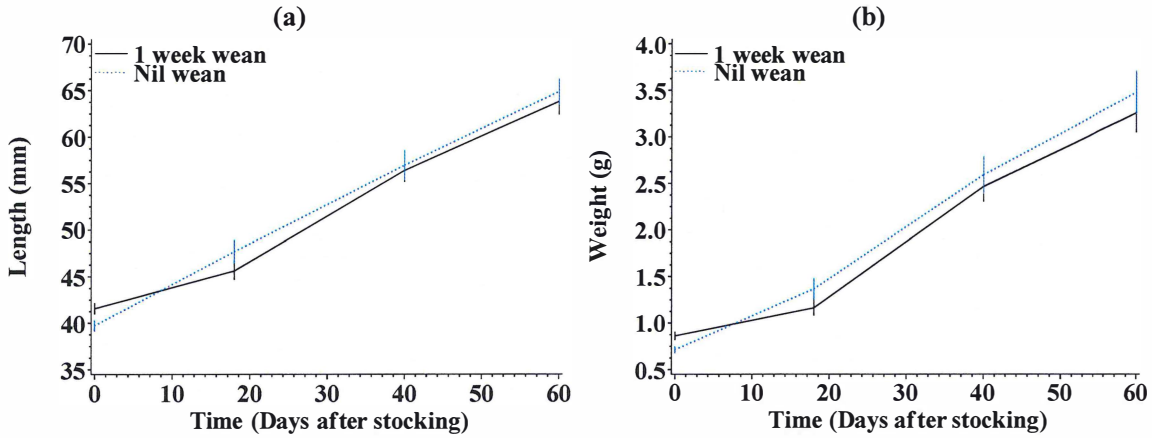

Fig. 3.8. Effect of a weaning duration on (a) length (mean ± s.e.) and (b) weight (mean ± s.e.), of Murray cod fingerlings during and after weaning.

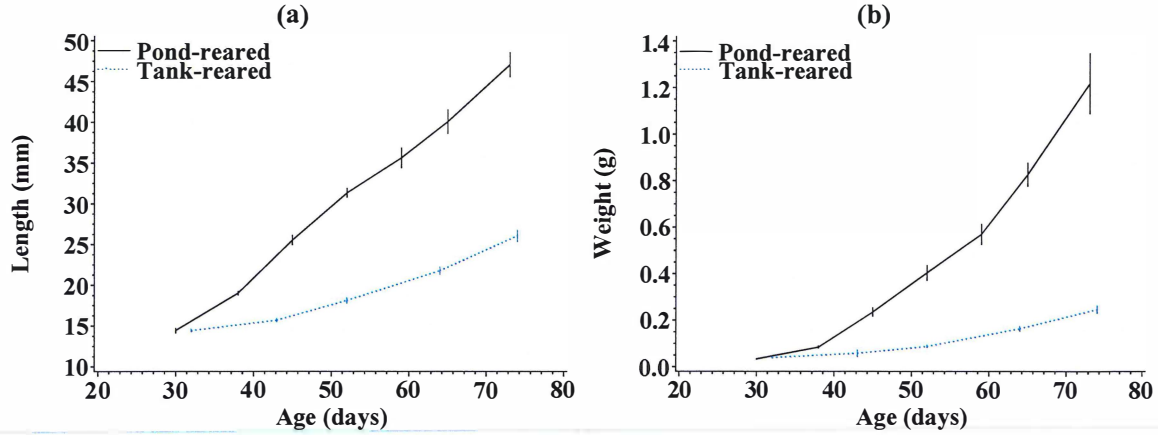

Fig. 3.9. Change in (a) length (mean ± s.e.) and (b) weight (mean ± s.e.) of juvenile pond-reared and tank-reared Murray cod.

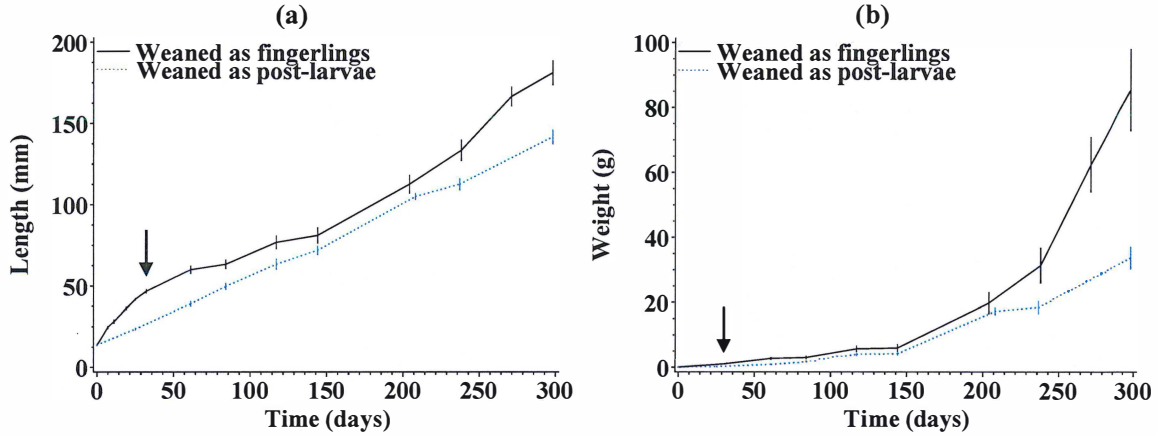

Fig. 3.10. Change in (a) length (mean ± s.e.) and (b) weight (mean ± s.e.) of juvenile Murray cod weaned as post-larvae and tank-reared, or pond-reared then weaned as fingerlings (Trial 1) (Arrow indicates commencement of weaning of pond-reared fingerlings).

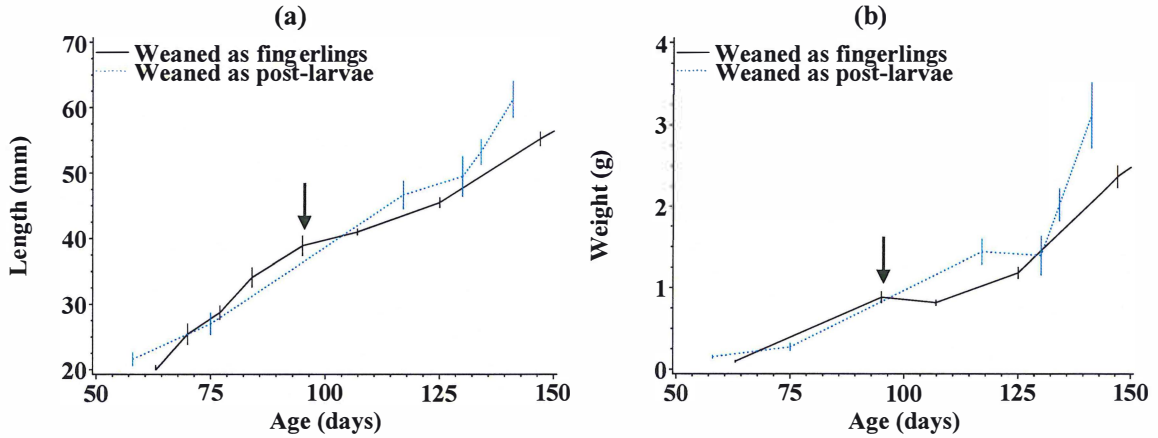

Fig. 3.11. Change in (a) length (mean ± s.e.) and (b) weight (mean ± s.e.) of juvenile Murray cod weaned as post-larvae and tank reared or pond-reared then weaned as fingerlings (Trial 2) (Arrow indicates commencement of weaning of pond-reared fingerlings)

Table 3.8. Initial and final weight, length, condition, specific growth rate (SGR) and survival rate of juvenile Murray cod reared under different conditions (values = mean ± s.e.).

Parameter		Treatment	
Pond reared v tank reared		*Tank-reared*	*Pond-reared*
Mean Length (mm)	- Initial	14.4 ± 0.09	14.5 ± 0.14
	- Final	26.0 ± 0.4	47.1 ± 0.7
Mean weight (g)	- Initial	0.038 ± 0.001	0.034 ± 0.001
	- Final	0.245 ± 0.008	1.22 ± 0.06
Condition	- Initial	1.26 ± 0.02	1.12 ±0.03
	- Final	1.39 ± 0.03	1.15 ± 0.02
Survival rate (%)		68 ± 3	95
SGR (%/day)		4.42 ± 0.28	8.36
Pond reared v tank reared – Trial 1		*Weaned as post-larvae*	*Weaned as fingerlings*
Mean Length (mm)	- Initial	13.61 ± 0.10	13.61 ± 0.10
	- Final	141 ± 2	181 ± 4
Mean weight (g)	- Initial	0.0419 ± 0.001	0.0419 ± 0.001
	- Final	33.5 ± 1.7	85.4 ± 6.3
Condition	- Initial	1.26 ±0.005	1.26 ±0.005
	- Final	1.16 ± 0.01	1.39 ± 0.03
SGR (%/day)		2.24	2.56
Pond reared v tank reared – Trial 2		*Weaned as post-larvae*	*Weaned as fingerlings*
Mean Length (mm)	- Initial	15.7 ± 0.15	19.9 ± 0.4
	- Final	61.2 ± 1.4	55.2 ± 0.5
Mean weight (g)	- Initial	0.052 ± 0.001	0.099 ± 0.005
	- Final	3.10 ± 0.2	2.36 ± 0.07
Condition	- Initial	1.23 ±0.03	1.20 ± 0.01
	- Final	1.32 ± 0.04	1.35 ± 0.01
Survival rate (%)		72	28
SGR (%/day)		3.70	3.32

During Trial 2, most mortalities of fish weaned as post-larvae occurred during the first 2-3 weeks of the weaning phase, and by the time these fish were 60 days old, the overall survival rate was 84% and mortalities had stabilised to less than 1%/week (Fig. 3.12). In contrast, survival of fingerlings from Pond 15, when compared to historical data (see section "Historical pond culture data") was low, with only 40% recovered from the pond at harvest (fish were 106 days old). Following harvest and during the weaning phase, mortalities increased, but by the third week following harvest, when fish were about 134 days old, the mortality rate had levelled off (Fig. 3.12). At termination of Trial 2 stocking density had reached 57 kg/m^3.

3.3.2 Effect of water temperature

SGR's (P=0.006), survival rates (P = 0.016) and feed rates (P<0.0001) of Murray cod reared at three different water temperatures were significantly different from each (Table 3.9). Growth of fish reared at 25°C was substantially greater than growth of fish reared at lower temperatures (Fig. 3.13). The final weight, final length and final condition of fish reared at the highest temperature were also significantly greater than for other temperatures (P<0.0001) (Table 3.9). SGR's for fish reared at the highest temperature (0.81 %/day) were nearly double that for fish reared at the lowest temperature (0.43 %/day), but SGR's for fish reared at the lower two temperatures were not significantly different from each other (Table 3.9). Survival rates of fish reared at the medium and high temperatures were not significantly different from each other, but were significantly greater than for fish reared at the lowest temperature. There were no clear reasons why the mortalities were highest in the low temperature treatment. Not surprisingly, feed rates increased significantly with increasing water temperature, but FCR's were not significantly (P=0.106) different between treatments (Table 3.9).

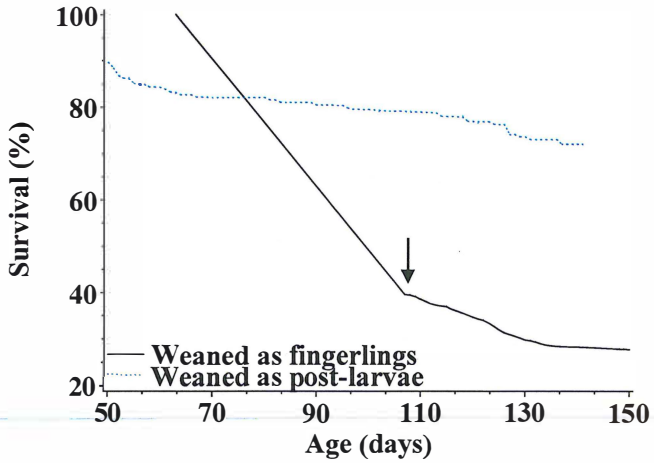

Fig. 3.12. Survival rates of Murray cod weaned as post-larvae and tank-reared or pond-reared then weaned as fingerlings (Arrow indicates commencement of weaning of pond-reared fingerlings).

Table 3.9. Initial and final mean weight, mean length, condition, specific growth rate (SGR), survival rate, FCR and feed rate of Murray cod reared at thee different temperatures (values = mean ± s.e.).

Parameter		Treatment*		
		Low temperature	*Medium temperature*	*High temperature*
Mean Length (mm)	- Initial	188 ± 1	186 ± 1	186 ± 1
	- Final	215 ± 2[a]	220 ± 2[a]	230 ± 2[b]
Mean weight (g)	- Initial	86.5 ± 1.8	84.2 ± 1.7	81.0 ± 1.4
	- Final	125.1 ± 3.3[a]	134.1 ± 4.2[a]	162.5 ± 3.7[b]
Condition	- Initial	1.28 ± 0.01	1.29 ± 0.01	1.26 ± 0.01
	- Final	1.24 ± 0.01[a]	1.24 ± 0.01[a]	1.31 ± 0.01[b]
Survival rate (%)		83 ± 4[a]	98 ± 2[b]	100[b]
SGR (%/day)		0.43 ± 0.03[a]	0.53 ± 0.03[a]	0.81 ± 0.03[b]
Feed rate (%/day)		0.75 ± 0.02[a]	1.06 ± 0.02[b]	1.26 ± 0.02[c]
FCR		1.75 ± 0.07	1.90 ± 0.02	1.54 ± 0.05

* Treatments with the same letter (superscript) are not significantly different from each other

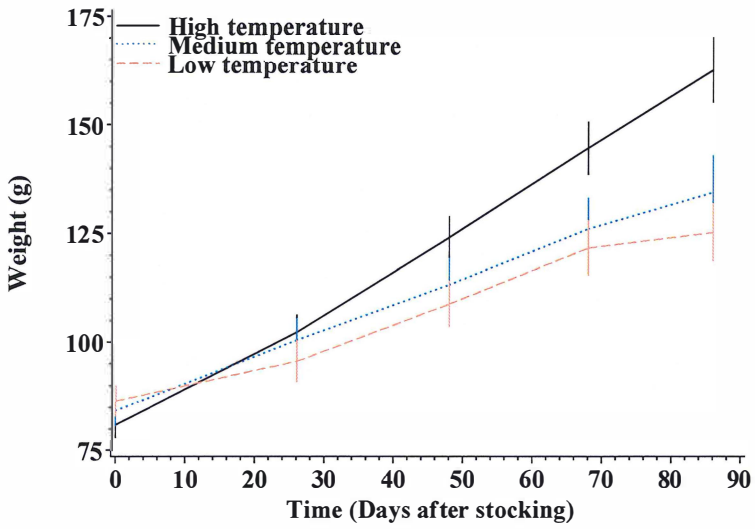

Fig. 3.13. Growth (mean weight ± s.e.) of Murray cod reared in tanks at three different temperatures (High temperature = 25°C. Medium temperature = 22.5°C. Low temperature = 20°C).

Analysis of water quality data showed that water temperature, DO and pH were significantly different between treatments (Table 3.10). Both pH and DO declined with increasing water temperature. Depressed water temperatures recorded in all treatments during the first and fifth weeks of the trial were due to a breakdown of the water heating system (Fig. 3.14).

Table 3.10. Water quality variables (mean with standard error in brackets) measured during the water temperature trial.

Parameter	Treatment/outlet*		
	Low temperature	*Medium temperature*	*High temperature*
Temperature (oC)	20.2 (0.12)[a]	22.5 (0.14)[b]	24.8 (0.18)[c]
DO (mg/L)	9.0 (0.08)[a]	8.6 (0.11)[b]	7.8 (0.12)[c]
pH	6.58 (0.09)[a]	6.54 (0.01)[ab]	6.50 (0.03)[b]
TAN (mg/L)	0.22 (0.04)	0.54 (0.15)	0.41 (0.11)
NO_2-N (mg/L)	0.004 (0.001)	0.005 (0.002)	0.006 (0.003)
Phosphate (mg/L)	0.12 (0.04)	0.34 (0.23)	0.30 (0.16)
Total Alkalinity (mg/L)	6.9 (0.4)	6.9 (0.3)	6.8 (0.3)

* Treatments with the same letter (superscript) are not significantly different from each other

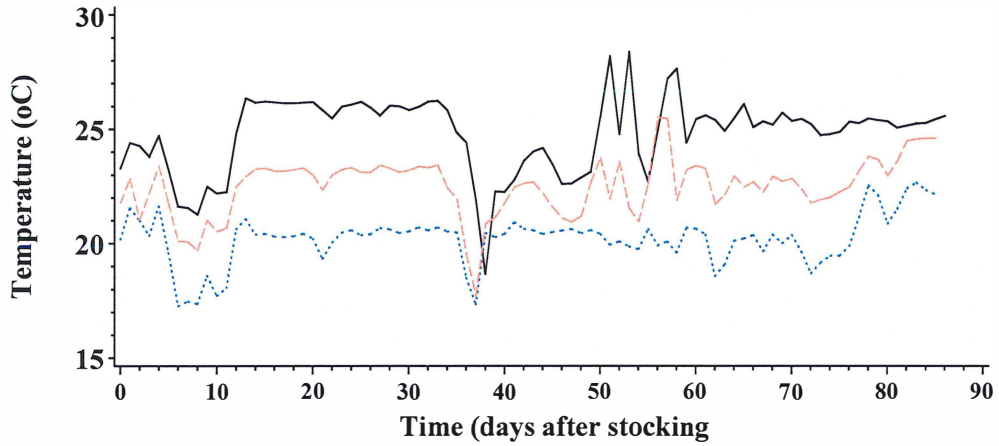

Fig. 3.14. Water temperatures recorded in tanks during a trial to determine the effects of different water temperatures on growth of Murray cod.

3.3.3 Commercial scale culture trials

3.3.3.1 Feed Trials 1 and 2

In Trial 1, Murray cod doubled their weight in less than 60 days. Diet type did not significantly affect SGR ($P=0.29$), however the final weight and condition of fish fed the salmon diet were significantly greater ($P<0.05$) than for fish fed the Deakin University (DU1) diet (Table 3.11, Fig. 3.15a).

During Feed Trial 2, Murray cod doubled their weight in 66 days and 78 days for fish fed the Deakin University (DU2) diet and the barramundi diet, respectively. Change in weight over time was not significantly different for diet (Fig. 3.15b), whereas change in length over time were significantly greater for fish fed the DU2 diet ($P=0.0126$). Further, both the final weight and final length of fish fed the DU2 diet were significantly greater than for fish fed the barramundi diet ($P<0.0001$) (Table 3.11). Overall, fish fed the DU2 diet had a greater SGR than did fish fed the barramundi diet, but this result was not statistically significant (Table 3.11).

In Trial 1, the feed rate for Murray cod fed the DU1 diet was significantly lower than for Murray cod fed the salmon diet ($P=0.022$), but FCR was not significantly different for diet type (Table 3.11). In Trial 2, fish fed the DU2 diet had a lower feed rate and FCR than did fish fed the barramundi diet, but these results were not statistically significant (Table 3.11). Feed rates in both trials varied substantially from day-to-day, and

tended to decline as fish increased in size (Fig. 3.16).

The proportion of feed not consumed by fish (uneaten feed collected from tanks daily) during Trial 2 was much greater for the barramundi diet than for the DU2 diet. The average daily amount of uneaten food was 12.3-17.6% (mean 15%/day) and 5.9-6.9% (mean 6.4%/day) for barramundi diet and the DU2 diet, respectively, with an overall average of 10.7%/day. These data were not collected during Trial 1. A more detailed description of nutritional aspects of these commercial scale feed trials are presented in De Silva *et al.* (2004).

Survival was not significantly different for diet type in either trial.

Water quality data recorded during these feed trials is summarised in Table 3.12.

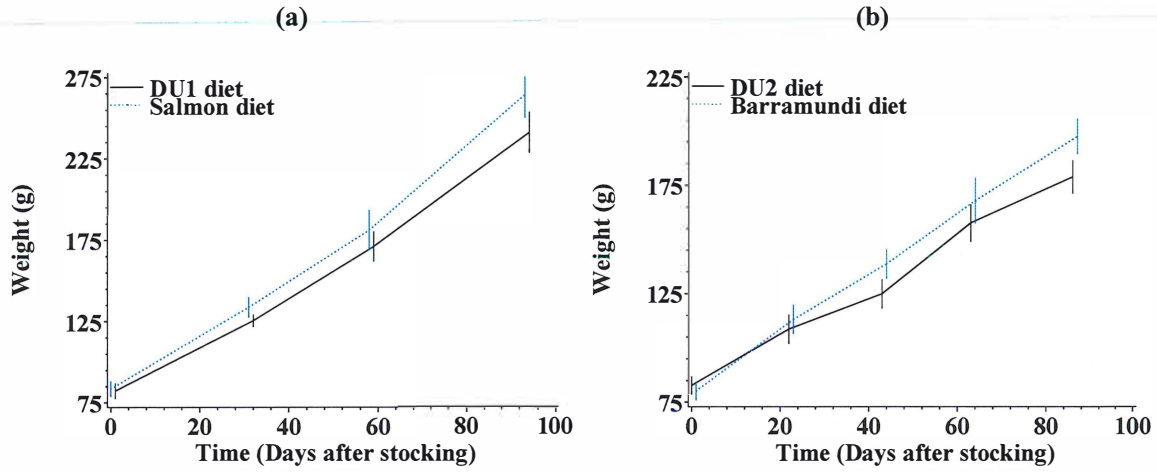

Fig. 3.15. Change in weight (mean ± s.e.) of Murray cod fed different diets under commercial production conditions. (a) Feed Trial 1 (b) Feed Trial 2

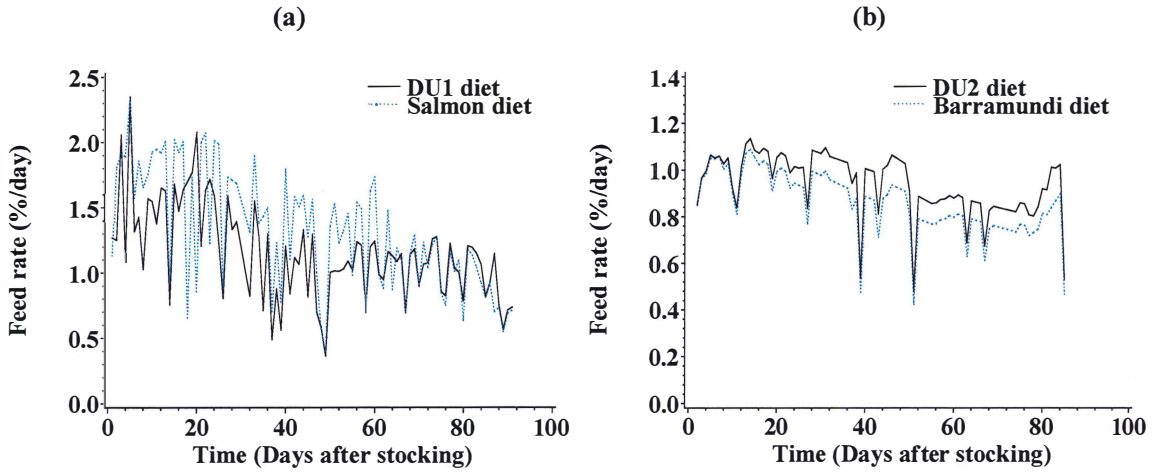

Fig. 3.16. Change in feed rate (daily mean) of Murray cod fed different diets under commercial production conditions. (a) Feed Trial 1 (b) Feed Trial 2.

Table 3.11. Initial and final mean weight, mean length, specific growth rate (SGR), survival rate, FCR and feed rate of Murray cod during two commercial scale culture trials (all values = mean ± standard error).

Parameter	Commercial feed Trial 1*		Commercial feed Trial 2*	
	Salmon grower	*Deakin University (DU1)*	*Barramundi high energy*	*Deakin University (DU2)*
Mean Length (mm)				
Initial	183 ± 2	182 ± 2	185 ± 1	183 ± 2
Final	263 ± 2	258 ± 2	235 ± 1[a]	243 ± 1[b]
Mean weight (g)				
Initial	83.5 ± 2.3	82.3 ± 2.3	82.8 ± 2.0	80.0 ± 2.0
Final	264.0 ± 7.0[a]	241.1 ± 6.2[b]	178.7 ± 3.8[a]	197.0 ± 4.1[b]
Condition factor				
Initial	1.36 ± 0.02	1.36 ± 0.02	1.29 ± 0.01	1.29 ± 0.01
Final	1.43 ± 0.01[a]	1.38 ± 0.02[b]	1.36 ± 0.01	1.35 ± 0.01
Survival rate (%)	99.6 ± 0.2	98.4 ± 0.4	99.5 ± 0.2	99.7 ± 0.1
SGR (%/day)	1.24 ± 0.06	1.16 ± 0.01	0.89 ± 0.05	1.05 ± 0.04
Feed rate (%/day)	1.24 ± 0.01[a]	1.09 ± 0.02[b]	0.94 ± 0.02	0.86 ± 0.03
FCR	1.00 ± 0.01	0.92 ± 0.03	1.09 ± 0.09	0.85 ± 0.04

* Treatments with the same letter (superscript) are not significantly different from each other.

Table 3.12. Water quality variables (mean with standard error in brackets) measured during two commercial scale culture trials.

Parameter	Commercial feed Trial 1		Commercial feed Trial 2	
	DU 1	*Salmon grower*	*System Inlet*	*System Outlet*
Temperature (oC)	25.5 (0.02)	25.2 (0.02)	20.0 (0.2)	20.1 (0.03)
DO (mg/L)			15.9 (0.1)	8.5 (0.1)
DO (% saturation)	105 (0.4)	106 (0.4)		79 (0.3)
pH	6.31 (0.01)	6.17 (0.01)	6.21 (0.04)	5.90 (0.01)
TAN (mg/L)	10.0 (2.7)	11.2 (2.6)	29 (0.6)	32 (0.6)
NO_2-N (mg/L)	2.1 (0.4)	2.4 (0.5)	0.34 (0.05)	0.31 (0.06)
NO_3-N (mg/L)	76 (5.4)	80 (6.4)	44 (2.5)	47 (2.9)
UIA (mg/L)			0.025 (0.002)	0.017 (0.002)
Total Phosphorus (mg/L)			12.2 (0.7)	13.9 (0.8)
BOD5 (mg/L)	14.3 (3.5)	8.0 (0.0)	8.7 (3.1)	19.2 (5.1)
Total Alkalinity (mg/L)	10.7 (2.2)	10.6 (2.4)	9.4 (0.9)	10.3 (1.1)
Turbidity (NTU)	7.4 (1.7)	7.0 (1.6)	5.2 (0.5)	10.5 (1.4)
Suspended solids (mg/L)			4.6 (0.5)	10.3 (1.1)

3.3.3.2 Growth of 1999 cohort

On 13th January 2000, AAP received a batch of juvenile Murray cod that were 20.8 – 40.8 mm (mean 29.9 mm) and 0.31 – 1.8 g (mean 0.82 g) in length and weight, respectively. At the time of stocking fish were about 65 days old. Fish were initially held in a quarantine RAS for up to 54 days. Fish were then transferred to 1,600 L tanks within the production facility. At the time of transfer, fish were 1.01 – 5.59 g (mean 3.15 g) in weight. When sufficient biomass was achieved fish were subsequently transferred to 15,000 L tanks for grow-out. These fish were 35-100 g (mean 60 g) in weight at the time of transfer.

Growth of Murray cod in various culture tanks during the study is presented in Fig. 3.17. Change in length of fish over time was more or less linear (Fig. 3.18a) whereas change in weight over time increased exponentially, and the weight range increased substantially (Fig. 3.18b). Apart from some juvenile fish that had condition values that exceeded 3.0 at the time of stocking, condition of fish, which ranged from 0.79 – 3.61 (mean 1.41) was fairly stable over much of the 300 day monitoring period (Fig. 3.18c). SGR's, which ranged from 0.26 – 4.36 %/day (mean 1.36% day), declined with increasing fish age (Fig. 3.18c).

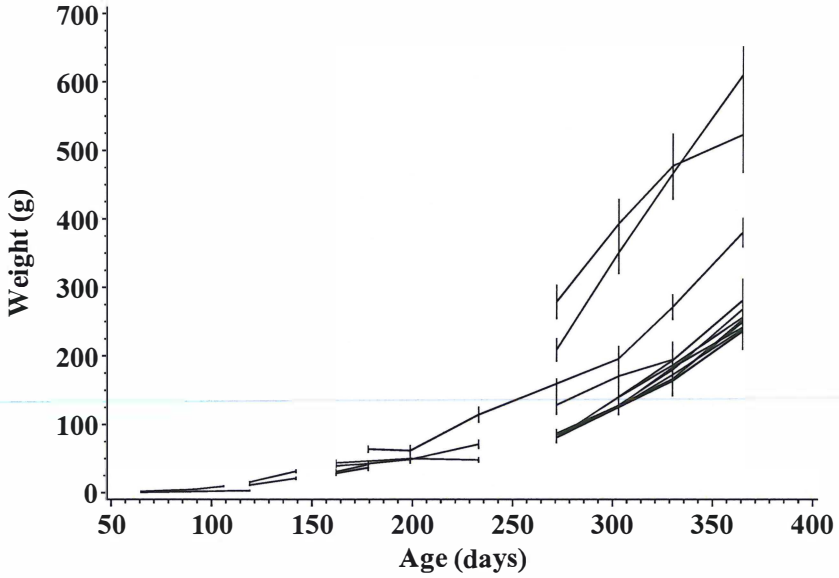

Fig. 3.17. Growth of Murray cod (mean weight ± s.e.) in different tanks in a commercial RAS.

After 300 days, when the fish had reached an average age of 365 days, fish length and weight ranged from 221 – 353 mm (mean 275 mm) and 150 – 785 g (mean 322 g), respectively (Fig. 3.18).

A summary of water data collected during the 300 days of this study is presented in Table 3.13.

3.3.4 Cage culture trials

3.3.4.1 Cage Culture Trial 1

Growth rates of fish during the trial were generally poor. Overall, fish increased in weight from 183 g to 274 g (SGR 0.33 %/day) (Table 3.14, Fig. 3.19). There was little difference in the growth of fish between the cages. Mortalities were highest in the more heavily stocked cage (69% survival after 55 days). The majority of mortalities corresponded with adverse environmental conditions, in particular high water temperatures and excessive algal blooms in the first month of the trial. During the first month, water temperature was consistently greater than 22°C and at times reached 30°C. During the 123 days of the trial, water temperature, pH, and DO ranged from 12-30°C (mean 19°C), 7.4-9.6 (mean 8.4) and 1.5-16.2 mg/L (mean 6.9 mg/L), respectively. Water temperature declined considerably during early March (Fig. 3.20), and by May, mean daily water temperatures were less than 15°C. Temperature data were not recorded between 7 February 2001 and 7 March 2001 due to failure of the datalogger. Feeding rates and FCR's were not determined for the trial due to and loss of appetite during stressful periods and changes in fish numbers in each cage. At termination of the trial fish were returned to PIRVic, Snobs Creek. Since 43% of these fish were infested with anchor worm (*Lernaea*) (Copepoda) (up to 5 parasites per fish), fish were placed in a quarantined recirculation system and treated with a constant salt bath (5 g/L) over a three-week period to eradicate the parasite.

3.3.4.2 Cage Culture Trial 2

After 86 days, growth rates and survival rates of Murray cod reared in cages ranged from 0.39-0.83%/day and 94-100%, respectively (Table 3.14). Some mortalities were the result of fish being trapped in netting covering the tops of the cages. Growth rates were greatest (up to 1.77%/day) during the first and second month of the trial, but tended to decline in latter months. In general, growth rates were greatest for "small" fish (mean 0.80%/day) and lowest for "large" fish (mean 0.46%/day) (Table 3.14, Fig.3.21a). Fish stocked at the lowest density (6.6 kg/m^3) generally had the lowest growth rates (Table 3.14, Fig.321b). By the end of the trial, stocking densities had reached a maximum of 43 kg/m^3 (high density cages).

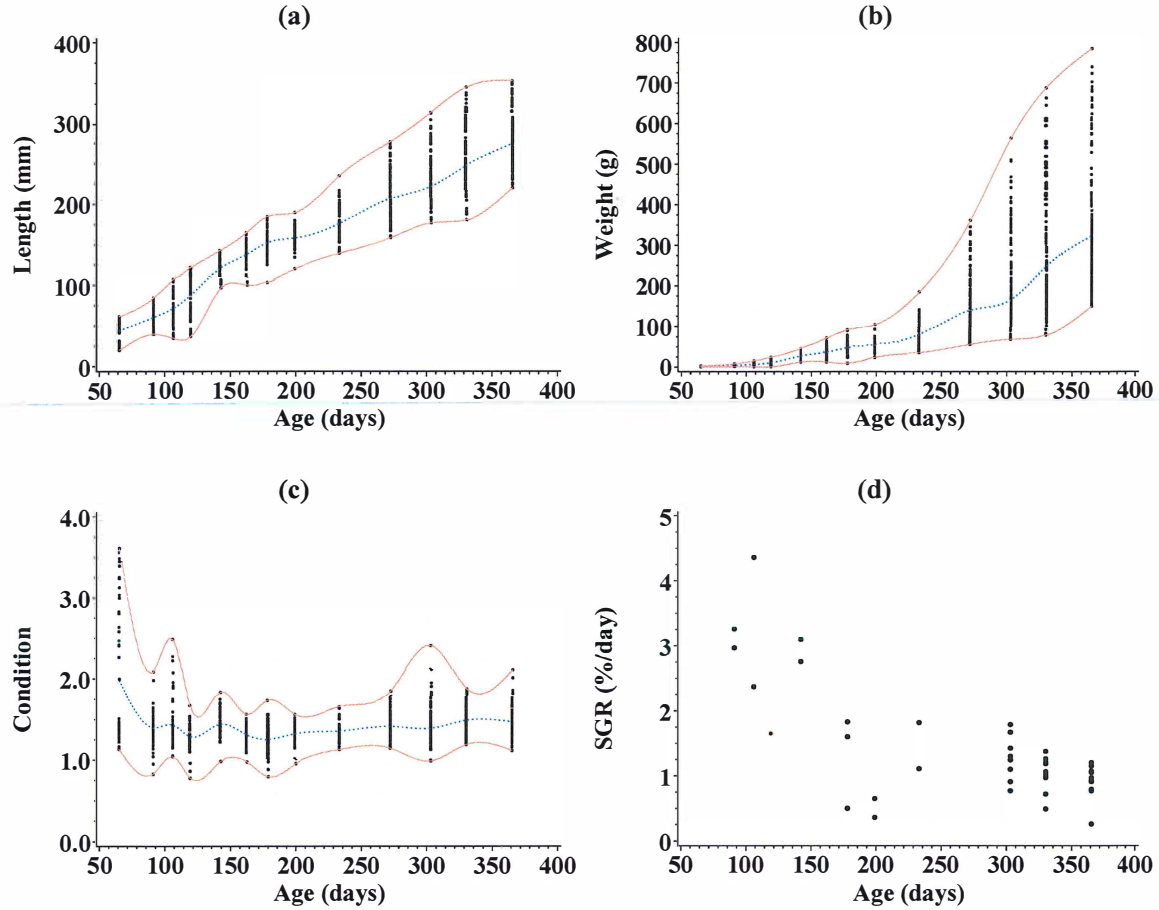

Fig. 3.18. Minimum, maximum and mean length (a), weight (b), condition (c) and SGR (d), of Murray cod reared in a commercial RAS (Dots = individual values, solid line = minimum and maximum, dashed line = mean).

Table 3.13. Water quality data collected during the grow-out of Murray cod in a commercial RAS.

Parameter	Mean	Range
Temperature (oC)	25.0.	16.7 – 27.7
DO (mg/L)	8.3	6.2 – 10.8
DO (% saturation)	105	32 – 172
pH	6.24	4.44 – 7.6
TAN (mg/L)	8.1	0 – 26.5
NO_2-N (mg/L)	1.06	0.003 – 6.0
NO_3-N (mg/L)	64	1 – 1,109
UIA (mg/L)	0.011	0 – 0.081
Total Phosphorus (mg/L)	11.5	0 – 20.1
BOD5 (mg/L)	15.8	8.0 – 25.0
Total Alkalinity (mg/L)	12.5	2.6 – 32.5
Turbidity (NTU)	6.8	0 – 16.0

FCR's ranged from 1.5-2.7 (mean 1.9). The were no signs of active feeding as a slow sinking diet was used and Murray cod did not feed at the water surface. Because there was a build-up of uneaten food on the bottom of some cages, feed rates were reduced in latter months of the trial. There was also a build-up of lattice algae (*Hydrodictyon reticulatum*) in all cages. Consequently, both uneaten food and lattice algae were periodically removed from the cages. During the first part of trial, mean daily water temperatures were between 20 and 23°C, but during April, temperatures declined appreciably and by May, were less than 16°C (Fig. 3.20).

Table 3.14 Initial and final mean weights, mean lengths, specific growth rates (SGR), survival rates, FCR's and feed rates of Murray cod during cage culture trials.

Parameter	Treatment					
Trial 1	*Low density*	*High density*	*Combined*			
Mean Length (mm)						
Initial	245 ± 4	249 ± 4	247 ± 3			
Final	257 ± 4	259 ± 4	280 ± 3			
Mean weight (g)						
Initial	182 ± 9	184 ± 10	183 ± 7			
Final	211 ± 14	219 ± 12	274 ± 11			
Condition factor						
Initial	1.22 ± 0.02	1.19 ± 0.03	1.20 ± 0.02			
Final	1.20 ± 0.03	1.24 ± 0.02	1.19 ± 0.02			
Duration (days)	55	55	123			
Survival rate (%)	87	69	59			
SGR (%/day)	0.26	0.31	0.33			
Trial 2	*Small size Low density*	*Small size Medium density*	*Small size High density*	*Medium size Low density*	*Medium size High density*	*Large size Medium density*
Mean Length (mm)						
Initial	209 ± 2	209 ± 2	209 ± 2	238 ± 2	238 ± 2	278 ± 3
Final	249 ± 3	256 ± 3	253 ± 5	267 ± 4	275 ± 4	315 ± 5
Mean weight (g)						
Initial	114 ± 3	114 ± 3	114 ± 3	182 ± 6	182 ± 6	343 ± 15
Final	219 ± 12	229 ± 10	233 ± 17	254 ± 18	305 ± 16	509 ± 26
Condition factor						
Initial	1.22 ± 0.02	1.22 ± 0.02	1.22 ± 0.02	1.33 ± 0.01	1.33 ± 0.01	1.57 ± 0.03
Final	1.40 ± 0.03	1.34 ± 0.02	1.38 ± 0.02	1.30 ± 0.04	1.43 ± 0.03	1.59 ± 0.02
Duration (days)	86	86	86	86	86	86
Survival rate (%)	98	97	94	100	98	98
FCR	1.71	1.5	1.61	2.68	1.88	2.21
SGR (%/day)	0.77	0.81	0.83	0.39	0.60	0.46
Feed rate (%/day)	0.86	0.85	0.85	0.89	0.87	0.89

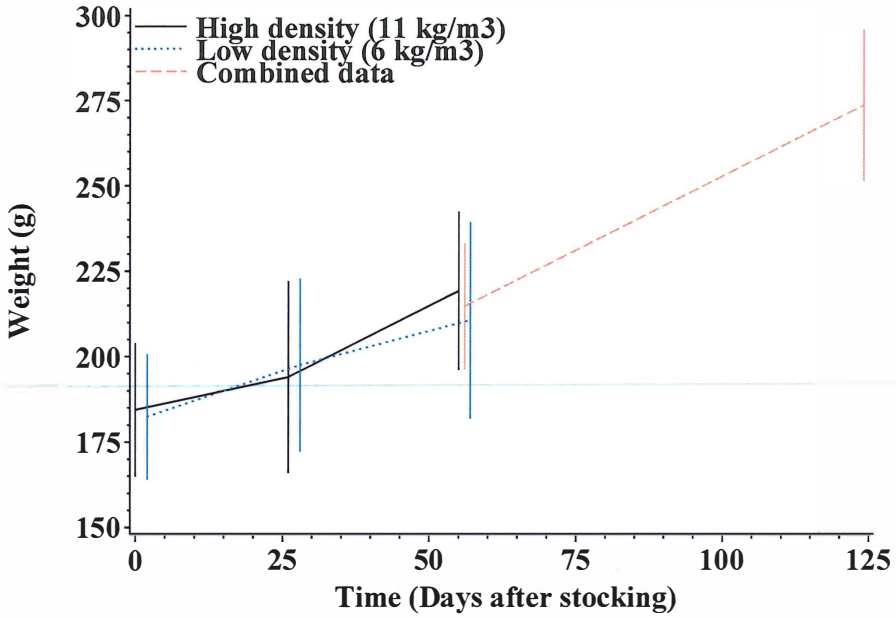

Fig. 3.19. Growth (mean weight ± s.e.) of Murray cod stocked into cages during Cage Culture Trial 1.

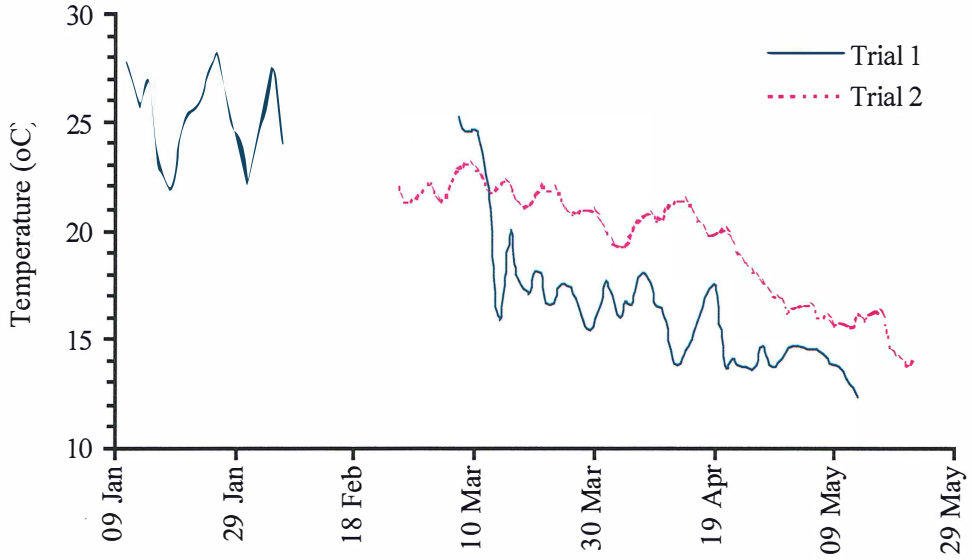

Fig. 3.20. Mean daily water temperature measured during two cage culture trials conducted in 2001 (Trial 1) and 2002 (Trial 2).

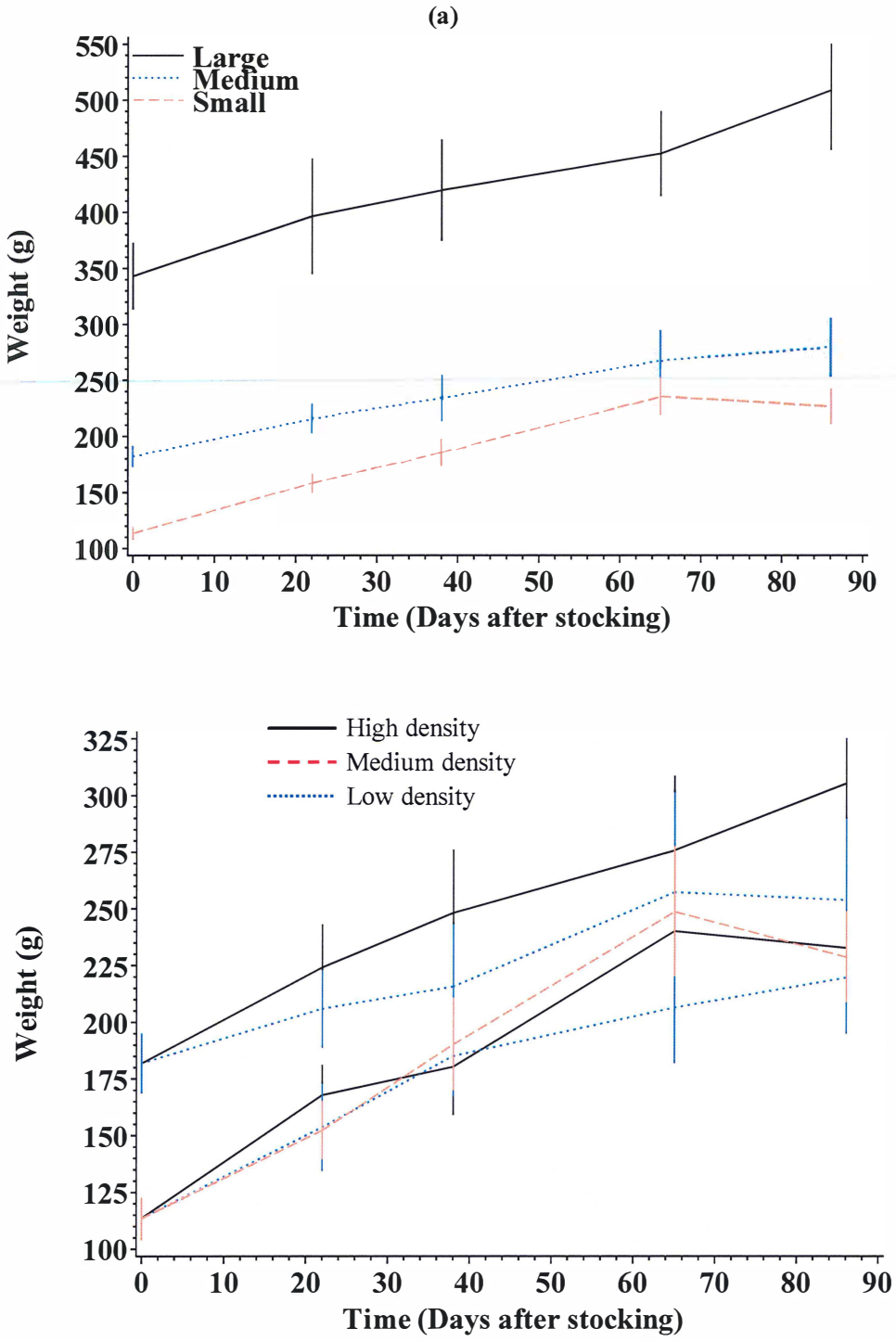

Fig. 3.21. Growth (mean weight ± s.e.) of Murray cod stocked into cages during Cage Culture Trial 2. (a) Growth at different initial stocking sizes (Large = 343 g, Medium = 182 g, Small = 114 g). (b) Growth at different stocking densities (high density = 25 kg/m^3, medium density = 16 kg/m^3, low density = 7 kg/m^3) for small and medium size classes of fish.

3.3.5 Data synthesis

Length, weight, condition, survival rates, SGR's, feed rates and FCR's, collected from all trials during the present study were synthesised to provide an overall summary of production information for Murray cod farmed under intensive culture conditions. Data were grouped according to fish size (Table 3.15).

The length-weight, length-age and weight-age relationships (determined by regression) for intensively farmed Murray cod (Fig. 3.22) were:

Log weight = 3.029 x Log length – 11.387 (adj. $R^2 = 0.998$, $P < 0.0001$)
Log length = 1.069 x Log age – 0.952 (adj. $R^2 = 0.861$, $P < 0.0001$)
Log weight = 3.484 x Log age – 15.613 (adj. $R^2 = 0.891$, $P < 0.0001$)

SGR's ranged from –1.86%/day (<1g fish) to 14.61%/day (<1 g fish) (Table 3.15). Most variation in SGR occurred in fish < 1 g in weight however, this group also had the highest mean SGR 5.06%/day. SGR's generally declined with increasing size (Fig. 3.23). The relationship between initial weight and SGR was:

Log SGR = -0.243 x Log initial weight + 0.500 (adj. $R^2 = 0.314$, $P < 0.0001$)

Growth of Murray cod was strongly influenced by water temperature (see also Section "Effect of water temperature"). SGR was positively correlated with water temperature for all size classes of fish (Fig. 3.24). Based on these values, Murray cod stocked into an intensive RAS as 1 g fingerlings and reared at a water temperature of 22-26°C, will reach a market size of 700g in 36-108 weeks (mean 63 weeks).

Fish reared in tanks generally had higher SGR's than fish reared in cages in ponds. Mean SGR's of fish with an initial mean weight of 150-500 g, were 0.92%/day (range 0.26-1.67%/day) and 0.52%/day (range 0.19-1.39 %/day) for tank-reared fish and cage-reared fish, respectively.

Mortality rates decreased with fish size (Table 3.15). Most variation in mortality rates occurred in fish less than 1 g in weight however, this group also had the highest mean mortality rate of 20% (Fig. 3.25). By the time fish had exceeded 50g in weight mortality rates were less than 5%. Based on these data, 59-88 % (mean 71%) of Murray cod, stocked into an intensive RAS as 1 g fingerlings survive to reach a market size of 700g.

Condition of fish, which ranged from 0.52 – 11.2 (mean 1.29) (Table 3.15), was related to the diet type. For example, fish fed a salmon grower diet (protein:lipid = 45:22) had a condition of 1.31-1.55 (mean 1.43), whereas fish fed a barramundi high energy diet (protein:lipid = 45:20) had a condition of 1.26-1.46 (mean 1.36). In the present study, condition was not significantly correlated to fish age (Table 3.15).

Feed rates ranged from 0.61-4.90 %/day (mean 1.82 %/day) (Table 3.15). Most variation in feed rates occurred in fish less than 1 g in weight, but the highest mean feed rate of 4.90%/day was observed in fish between 1-5 g in weight. Feed rates declined with increasing fish size (Fig. 3.26), which supports results from previous trials undertaken during the present study (Fig. 3.16). The relationship between initial weight and feed rate was:

Log feed rate = -0.277 x Log initial weight + 1.338 (adj. $R^2 = 0.835$, $P < 0.0001$)

Feed rates were positively correlated with water temperature for all size classes of Murray cod (Fig. 3.26). For fish with a initial mean weight of 150-500 g, feed rates of fish reared in tanks (mean 0.92 %/day, range 0.61-1.08 %/day) were generally similar to fish reared in cages in ponds (mean 0.90 %/day, range 0.85-0.96 %/day). During the present study, Murray cod accepted both floating and sinking diets, but appeared to prefer the latter. Size of pellets offered to Murray cod during the present study was dictated by size of fish (Fig. 3.27)

FCR's were highly variable, ranging from 0.52 to 6.08 (mean 1.73), and tended to decline with increasing fish size. Fish with an initial mean weight <5 g had a mean FCR of 1.92-2.25 whereas fish with an initial mean weight >5 g had a mean FCR of 1.21-1.63 (Table 3.15).

Stocking densities during the present study ranged from <1 to 182 kg/m^3 in tanks and 5.5 – 43 kg/m^3 in cages in ponds. Highest stocking densities (72 – 182 kg/m^3) occurred during commercial scale feed trials in intensive RAS in which water entering culture tanks was supersaturated with DO. In other tank-based trials in which water was aerated only, the maximum stocking density observed was 70 kg/m^3.

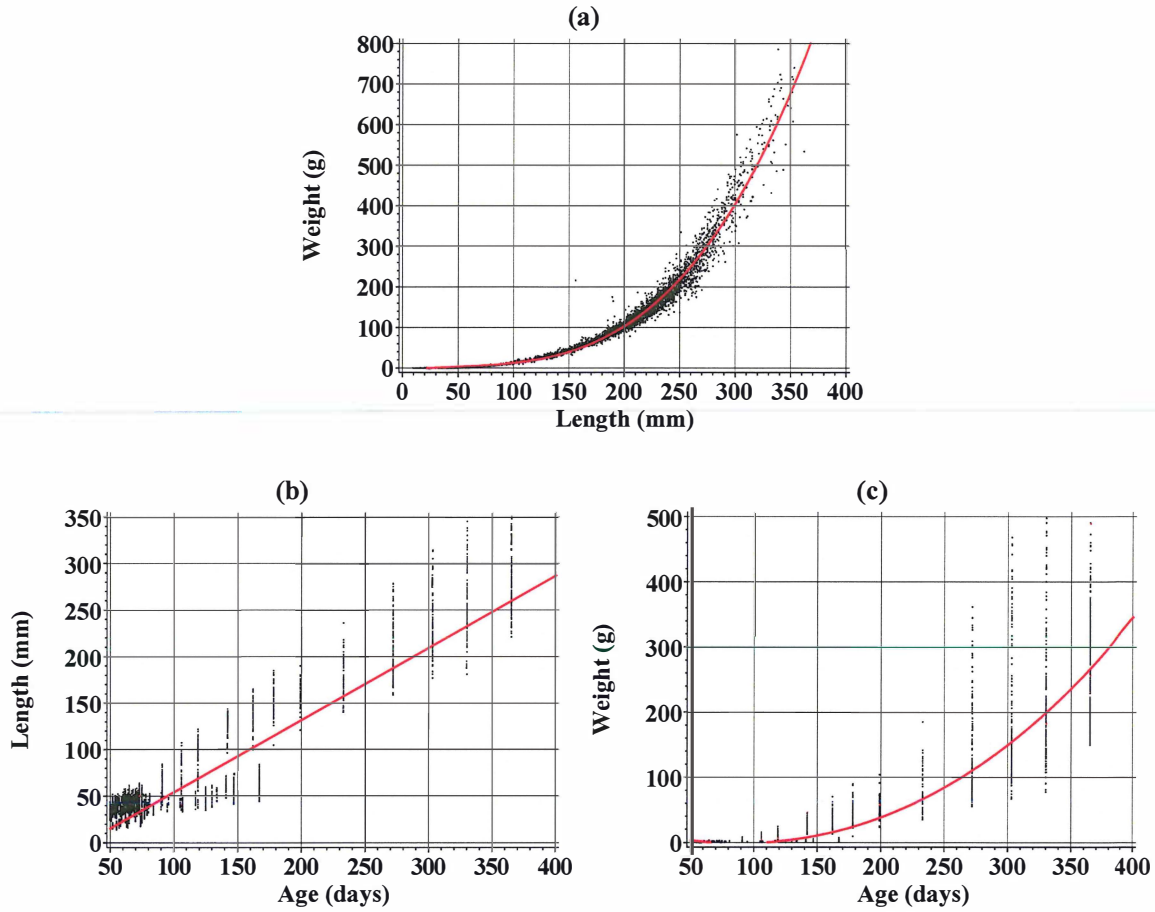

Fig. 3.22. Length-weight (a), age-length (b) and age-weight (c) relationships for intensively farmed Murray cod (see text for regression equations).

Table 3.15. Summary of production parameters (mean and range) for intensively farmed Murray cod.

Parameter	Fish weight (g)*					
	Overall	<1	1-5	5-50	50-150	150-500
SGR (%/day)	2.27	5.06	1.23	1.28	0.95	0.73
	(-1.86 - 14.61)	(-1.86 - 14.61)	(-1.23 - 6.24)	(-0.10 – 3.40)	(-0.16 – 1.95)	(-0.4 – 1.67)
Mortality rate (%)	47	26	20	7.5	2.5	1.5
	(23-82)	(13 – 70)	(5 – 30)	(5 – 10)	(1 – 5)	(1 – 2)
Condition	1.29	1.26	1.24	1.33	1.31	1.41
	(0.52 – 11.2)	(0.52 – 11.2)	(0.77 – 3.48)	(0.79 – 1.90)	(0.88 – 1.86)	(0.94 – 2.59)
Feed rate (%/day)	1.82	3.19	4.23	0.86	1.06	0.91
	(0.61 – 4.90)	(2.10 – 4.00)	(3.33 – 4.90)	(0.81 – 0.94)	(0.72 – 1.72)	(0.61 – 1.08)
FCR	1.73	1.92	2.25	1.25	1.63	1.21
	(0.52 – 6.08)	(0.90 – 5.00)	(0.86 – 5.44)	(1.05 – 1.63)	(0.52 – 6.08)	(0.83 – 3.19)

* Mean weight at start of measurement period

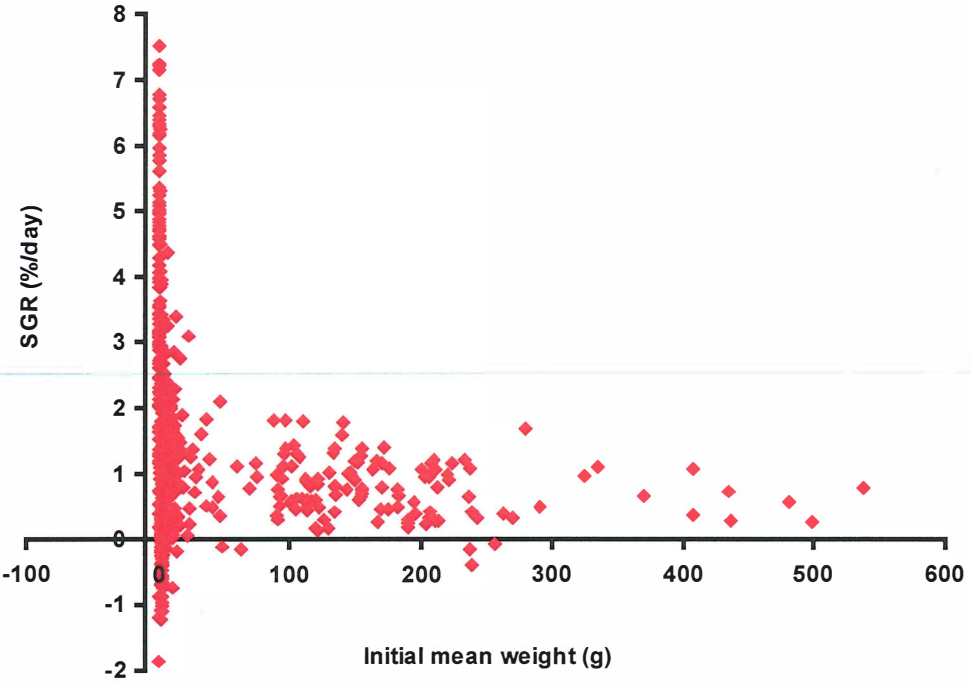

Fig. 3.23. Relationship between initial mean weight and SGR of intensively farmed Murray cod.

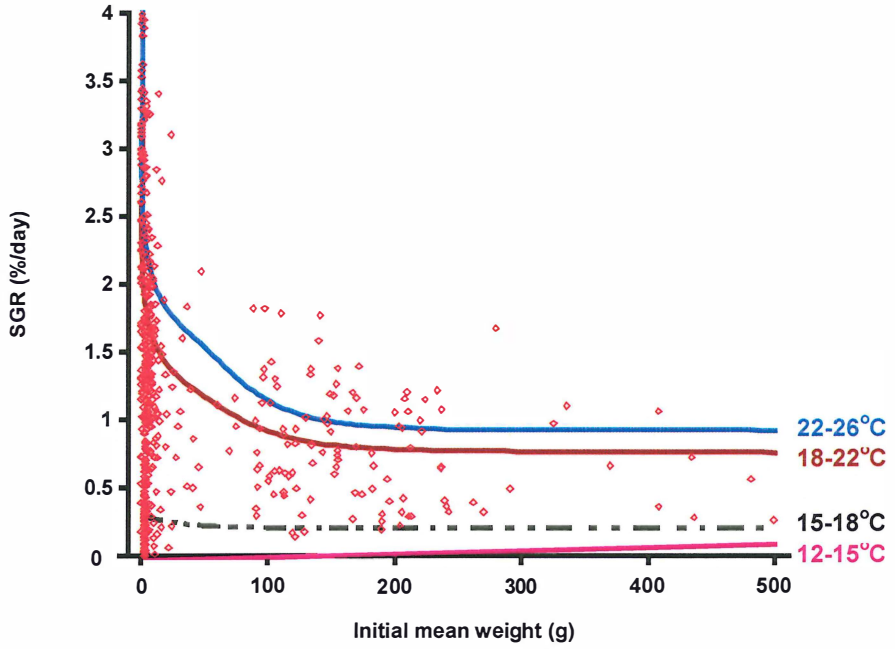

Fig. 3.24. Relationship between initial mean weight, water temperature and SGR of intensively farmed Murray cod.

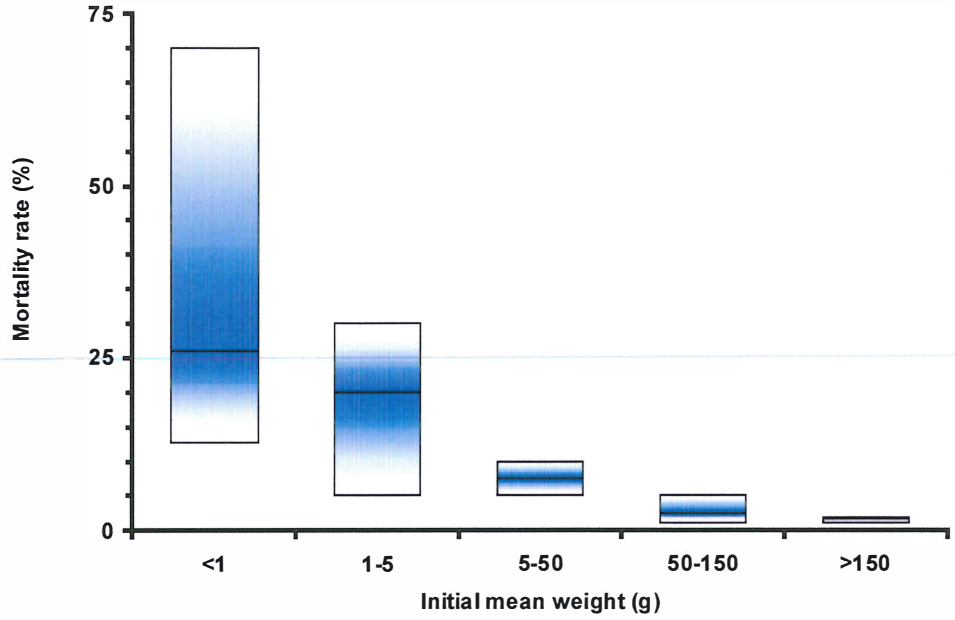

Fig. 3.25 Relationship between initial mean weight and mortality rate (mean ± range) of intensively farmed Murray cod.

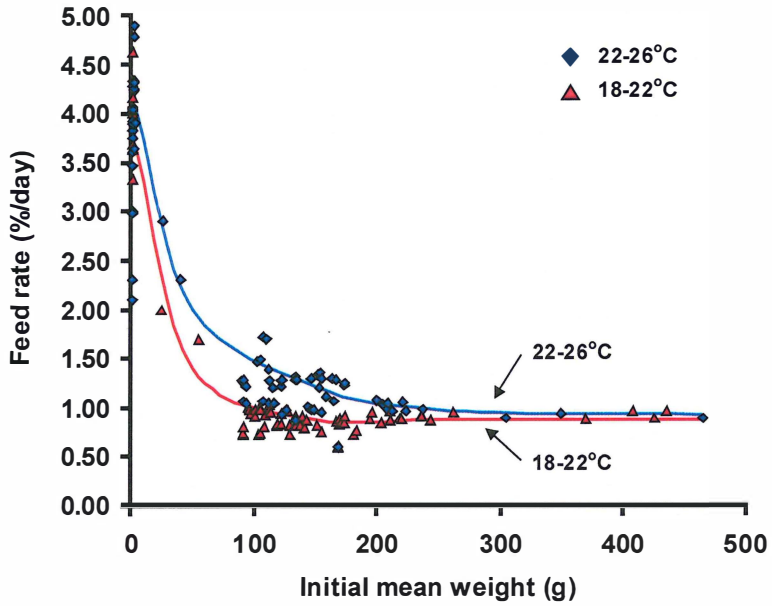

Fig. 3.26. Relationship between initial mean weight, water temperature range and feed rate of intensively farmed Murray cod.

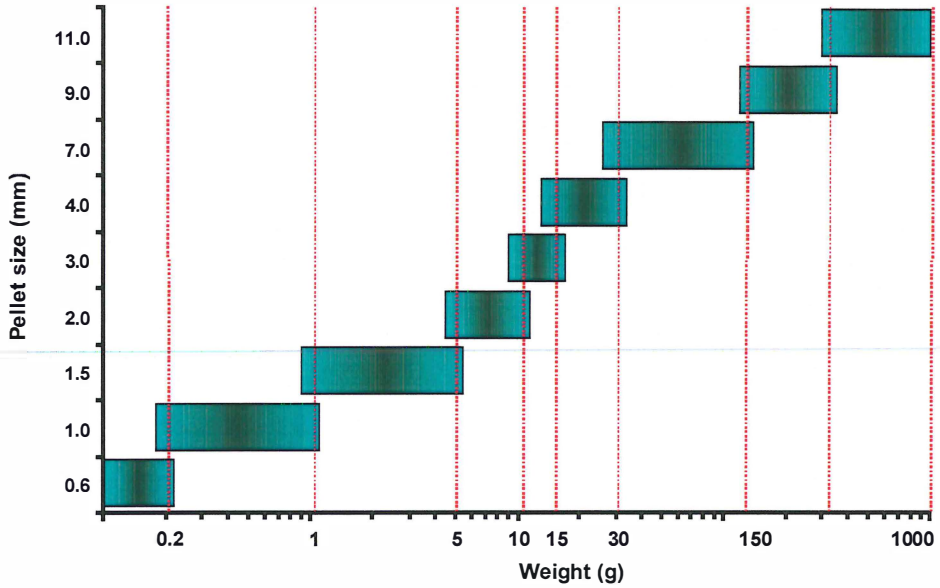

Fig. 3.27. Size of feed pellets suitable for different sizes of Murray cod

3.4 Discussion

3.4.1 Fingerling production

Initial acclimation of new stock to aquaculture conditions, and initial stages of feeding (weaning) have been identified as a critical phase in the successful culture of several species of fish (Sheikh-Eldin *et al.* 1997; Ingram *et al.* 2001a; Shields 2001; Ljunggren *et al.* 2003). Indeed, this phase can influence growth and survival throughout subsequent culture phases as non-acceptance of food, stress, cannibalism and disease may cause loss of growth and mortality. The present study found that diet type, duration of the weaning phase and stocking density significantly affected the growth of Murray cod post-larvae, but not survival. The most appropriate diet for weaned Murray cod post-larvae was found to be a 0.4-0.6 mm salmon starter diet (protein:lipid 50:15), which is commercially available and currently being used by industry. The present study showed that, despite low survival rates, a proportion of Murray cod post-larvae would accept an artificial diet without the need of a weaning phase. However, few finfish species can be reared exclusively on artificial diets from first feeding (Jones *et al.* 1993), and several studies into the weaning of larval fish have shown that growth and survival rates are greater in larvae weaned gradually (Sheikh-Eldin *et al.* 1997; Bengtson *et al.* 1999; Daniels and Hodson 1999). Therefore, a gradual weaning phase is recommended for Murray cod post-larvae to ensure optimal growth and survival.

In the present study, conflicting results were obtained in two stocking density trials during the weaning of post-larvae; final length of post-larvae was greatest at the highest density (30 fish/L), and the lowset density (10fish/L) in Trial 1 and Trial 2, respectively. Houde (1975) and Sheikh-Eldin *et al.* (1997) found that both growth and survival of larval sea bream (*Archosargus rhomboidalis*) and Macquarie perch (*Macquaria australasica*), respectively, were greatest at lower stocking densities ($\leq$ 8 fish/L). At higher densities, competition for food and antagonism between fish are increased, which may have affected growth and survival during and after the weaning phase. However, at higher densities, development of aggressive behaviour amongst fish may be inhibited (Wallace *et al.* 1988). Krise and Meade (1986) indicated that stocking densities up to 35 fish/L were appropriate for the intensive culture of walleye (*Stizostedion vitreum*) larvae. Further research is required to identify appropriate stocking densities for the weaning of Murray cod post-larvae.

Weaning techniques can also greatly influence the success of weaning fingerlings onto artificial diets (Ljunggren *et al.* 2003). The present study showed that growth is affected by the condition of fingerlings and stocking density at commencement of weaning, whereas survival is not affected. These results indicate that the condition of fingerlings at commencement of weaning strongly influences the success of transition onto an artificial diet. Fish that are in poor condition at the time of harvest from rearing ponds, or commencement of weaning following harvest is delayed, will be more difficult to wean. Fewer fish are likely to wean onto the artificial diet and both survival and growth rates are likely to be depressed when compared to fish that are in good condition and immediately placed onto a weaning schedule at the time of harvest.

In the present study, cannibalism was found to occur during the weaning of fingerlings, and may have contributed to the relatively low survival rates (50-71%) observed in some trials. Typically, survival rates between 70% and 95% would be expected to be achieved during the weaning of fingerlings (initial weight 1 g) (Table 3.15; Ingram *et al.* 2001b). Cannibalism was not observed in Murray cod post-larvae in the present study. The age at which Murray cod become aggressive and cannibalistic is not known. Japanese flounder (*Paralichthys olivaceus*) become aggressive about 39 days after hatching, which coincides with completion of metamorphosis (Sakakura and Tsukamoto 2002). Factors that may affect the incidence of cannibalism in Murray cod include fish density, starvation and size variation. During weaning trials in the present study, growth rates were greatest in fingerlings stocked at the lowest density (7 kg/m^3) whereas survival was not affected. Yet, Zakes (1997) found that as densities of pikeperch (*Stizostedion lucioperca*) fry were increased, natural deaths increased but losses due to cannibalism decreased, concluding that quite high fish densities can be used when rearing on a larger scale. Currently, the impacts of cannibalism in commercial Murray cod operations is avoided by stocking at high densities and grading fish every 2-4 weeks during the first few months of grow-out (Ingram and Lawson 2004).

Different fish roe types have been successfully used as a starter diet for weaning fish, especially *Anguilla* spp. (Heinsbroek 1991; De Silva *et al.* 2001), and the type (and chemical composition) of fish roe can influence the success of weaning (De Silva *et al.* 2001). However, carp roe has already been found to be a useful starter diet for Murray cod fingerlings in commercial operations. Based on the results from the present study, and consultation with industry, best practice weaning guidelines have been developed for fingerling Murray cod (Ingram *et al.* 2001b). These guidelines are intended as an extension tool to industry to improve survival and growth of Murray cod during this critical period and to increase overall productivity of Murray cod culture.

Apart from the factors discussed above, other factors that may contribute to weaning success of both post-larvae and fingerlings include: stress associated with handling, harvest and transport; food density; acceptability, palatability and digestibility of artificial feeds; and environmental conditions such as temperature, tank colour and light intensity (Houde 1975; Krise and Meade 1986; Colesante 1989; Jones *et al.* 1993; Kuipers and Summerfelt 1994; Denson and Smith 1996). Further research into the weaning of post-larvae and fingerlings of Murray cod may need to investigate at least some of these factors.

The present study demonstrated that both post-larvae and fingerling Murray cod can be successfully weaned onto an artificial diet relatively easily when compared to closely related percichthyids. For example the Chinese mandarin fish (*Siniperca chuatsi*), which will not accept inanimate or artificial feeds is cultured using solely live fish as food (Liang *et al.* 2001). The inability to wean golden perch (*Macquaria ambigua*) onto artificial diets has been identified as a major impediment to production of this species (Herbert and Graham 1999). However, it is unclear as to which method, weaning as post-larvae or weaning as fingerlings, is most appropriate for the cost-effective production of Murray cod seedstock for grow-out purposes. Over the first 2-3 months, survival rates are comparable but variable for both methods. Growth rates were greatest in Murray cod reared in ponds, and pond-reared fish were generally larger than fish of a similar age that were weaned as post-larvae and reared in tanks. However, in the month following harvest and weaning, the growth of pond-reared fingerlings declined while the growth of fish weaned as post-larvae was maintained to the point where these fish exceeded the size of pond-reared fish of a similar age that were weaned as fingerlings. These results indicate that pond-reared fingerlings require a period of time to acclimate to the relatively new and artificial environment of aquaculture tanks, and a new, artificial, diet. Bio-economic analyses, particularly taking into account costs associated with infrastructure and labour associated with these two methods, will need to be undertaken to determine the most cost effective option for production of weaned Murray cod seedstock.

3.4.2 Grow-out

Synopsis of data collected during the present study, has provided key production information, growth, survival, fish condition, feeding rates, and food conversion ratios, for the grow-out of Murray cod to a minimum market-size (>700 g). In the present study, SGR's which ranged from –1.9%/day to 14.6%/day (mean 2.3%/day), was negatively correlated with fish age and positively correlated with water temperature. For fish stocked into a RAS as 1 g fingerlings, between 59-88 % (mean 71%) are expected to survive to a market size of 700g. Based on these values, in a commercial operation, for every 1,000 fingerlings (1g/fish) stocked into a RAS, operating at a temperature between 22-26°C, between 413 and 616 kg (median 500 kg) of Murray cod (at 700 g/fish) are harvested within 108 weeks of stocking, though some individual fish will reach a market size within 36 weeks.

Successful aquaculture in RAS requires that fish be reared at high densities. For example, red tilapia (hybrid of *Oreochromis mossambicus* and *O. niloticu*) and European eels (*Anguilla anguilla*) have been farmed at densities up to 200 kg/m^3 and in excess of 300 kg/m^2, respectively (Suresh and Kwei Lin 1992; Anon 1998). The current study and work by Boreham *et al.* (2004) indicate that, under appropriate conditions, such as high DO and low ammonia concentrations, Murray cod may be cultured at high densities. In the present study, stocking densities up to 182 kg/m^3, whereas higher densities, in excess of 280 kg/m^3, have been reported by industry for Murray cod culture (O'Sullivan 2003).

The present study showed that growth of Murray cod was significantly affected by water temperature. Temperatures around 25°C provided for greatest growth rates in cultured Murray cod, whereas temperatures below about 16°C, growth was negligible. Similarly, feed rates were also strongly influenced by temperature for all size classes of Murray cod. However, further trials are required to determine production performance of Murray cod at temperatures above 25°C, to identify upper temperature limits and thus the optimal temperature range for the culture of Murray cod.

Water quality data collected during the present study have been used to develop water quality guidelines for the intensive production of Murray cod in RAS (Boreham *et al.* 2004), and a more detailed description of the effects of water quality on the health of Murray cod is provided in Ingram *et al.* (2004b).

RAS have been widely used to commercially farm a variety of species, including anguillids, tilipias, trout, catfish and silver perch (Suresh and Kwei Lin 1992; Larkin 2000; Eding and Kamstra 2001), and appear to be appropriate for the intensive farming of Murray cod. A standard feature of RAS is that a substantial proportion of the water utilised in the culture units (mostly tanks) is passed through a water treatment system and reused, which saves both water and power for heating and cooling (Timmons and Losordo 1994). Advantages of RAS include compactness (small footprint), biosecurity, high-density production and their ability to control the environment in which the animals are reared. RAS are considered more environmentally sound than open, flow-through aquaculture systems especially in terms of waste production and management (Mayer and McLean 1995). RAS allow for concentration and therefore substantial reductions in the volume of wastewater discharged, removal of substantial quantities of suspended solids and associated nutrients by microscreen filtration processes, and conversion of some nutrients via biofiltration (Rosenthal and Black 1993; Piedrahita 1994; Mayer and McLean 1995). Culture of fish at high densities in RAS requires close monitoring of water quality, especially DO, pH, TAN (and unionised ammonia) and nitrite to ensure that critical levels are not reached or exceeded. Stocking fish in RAS at high densities can expose stock to constant, elevated concentrations of ammonia and/or nitrite, which can suppress growth (Weirich *et al.* 1993; Hrubec *et al.* 1996).

3.4.3 Cage culture

Culture of fish in cages is widely practiced for many species (Liao and Lin 2000) and in inland waters of Australia, significant numbers of barramundi are farmed in cages (Gooley *et al.* 2000). Results from the present study indicate that Murray cod, at least stocked as "young-of-year" can be successfully reared in cages. However, SGR's (0.26-0.83%/day) were generally lower than for similar-sized Murray cod reared intensively in tanks (mean 0.95%/day). The poor survival rates (59%) and growth rates (0.26-0.33%/day) in the first cage culture trial were attributed to poor water quality associated with the presence of a large algal bloom in the pond and high water temperatures (up to 30°C). Further, infestation by *Lernaea* during the trial may have stressed fish and affected growth. Light infestations of *Lernaea* may not be life threatening to Murray cod but heavy infestations may lead to debilitation (Rowland and Ingram 1991). Problems

encountered during the first cage culture trial did not occur during the second cage culture trial in which greater survival rates (>94%) and SGR's (0.39-0.83%/day) were obtained.

Stocking density can affect growth and survival in cages (Konikoff and Lewis 1974). However, growth of Murray cod did not appear to be affected by stocking density in the present study. At termination of the second cage culture trial, stocking densities reached a maximum of 43 kg/m^3. Upper stocking density limits for the cage culture of Murray cod have yet to be determined, but may well be considerably higher than this. Silver perch reared in cages, which were adjacent to aerators, have achieved good growth at densities up to 90 kg/m^3 (S. Rowland *pers. comm.*). The highest density recorded for Murray cod held in aerated tanks during the present study was 70 kg/m^3.

Wild Murray cod characteristically have a pale green to creamy yellow background colour that is overlaid by an olive green to dark green mottled to marbled patterning. In contrast, Murray cod reared in intensive recirculation systems typically are considerably darker in appearance than wild fish (Larkin *et al.* 2004). Murray cod used in the second cage culture trial were sourced from a RAS at PIRVic, Snobs Creek and at termination of this trial, harvested fish were compared with similar sized fish from the RAS at PIRVic, Snobs Creek. Over the 12 week period that Murray cod were reared in the cages, their body colouration had reverted to that more characteristic of wild Murray cod (Plate 3a). Holding Murray cod in cages prior to marketing may be a useful means of value-adding to the product as fish acquire an appearance similar to wild Murray cod, which could be more appealing to some buyers at the market place (Larkin *et al.* 2004). Indeed, some farmers are already using ponds as part of the grow-out phase. Fish that have been over-wintered in RAS are grown through to market size in earthen ponds during the warmer months of the year (Ingram and Lawson 2004).

Declining SGR's observed in Murray cod in cage culture trials over time were attributed to declining water temperatures. Production of Murray cod in cages under ambient conditions, will be largely limited to the warmer months as growth of Murray cod at low temperatures (<16^{o}C) is limited. Further, Forster (1999) found that 1g Murray cod stocked into farm dams in northern NSW take three years to reach a size of 2-3 kg

Although Murray cod fingerlings are routinely reared in earthen ponds (Rowland 1986a; 1986b; 1992; Ingram 2001), no data is currently available on the pond- or cage-growout of Murray cod from the fingerling stage. Further, issues of weaning Murray cod in ponds and cages has yet to be addressed.

3.4.4 Conclusions

The present study has considerably broadened the knowledge on the culture of Murray cod particularly the weaning of post-larvae and fingerlings, and grow-out in tanks and cages under controlled and ambient conditions, respectively. The preliminary results presented here indicate that this species performs well especially under intensive culture conditions, as indicated by excellent growth rates, survival rates and FCR's at high stocking densities. These data have been used to determine the economic viability and profitability of Murray cod aquaculture under various production scenarios (Rawlinson 2004).

3.5 Acknowledgments

The author wishes to thank the following persons for their contributions to the project. Professor Sena De Silva and Dr. Rasanthi Gunasekera (Deakin University, Warrnambool) provided assistance and advice on nutritional aspects. Staff at PIRVic, Snobs Creek, especially Rod Mission, Jen Dobson, Peter Grant, Peter Ryder and Paul Petritis for providing assistance with on-site trials. Geoff Gooley from PIRVic Snobs Creek is especially thanked for advice throughout the study. Mr Denis Lovric (Deakin University) assisted with some trials. Special thanks are extended to Australian Aquaculture Products (Euroa) and staff (Roger Camm, Steve Vidler and Lynton Fisher), Peter and Carla van Lierop (Alexandra Fish Farm, Alexandra) and Roger Munzel (Torrumbarry silver perch farm) for providing access their facilities and for their generous support and assistance during *in situ* trials. The financial support of Fisheries Research and Development Corporation and Fisheries Victoria, Department of Natural Resources and Environment for this project is gratefully acknowledged.

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4 MURRAY COD NUTRITION

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De Silva, S.S., Gunasekera, R.M., Collins, B., Abery, N., Ingram, B.A. and Gooley, G.J. (2004). Murray cod nutrition. In: *Development of Intensive Commercial Aquaculture Production Technology for Murray cod* (ed. B.A. Ingram and S.S. De Silva), pp 67-111. Final Report to the Fisheries Research and Development Corporation (Project No. 1999/328). Primary Industries Research Victoria, DPI, Alexandra, Victoria, Australia.

CONTENTS

4.1 Introduction

Murray cod, *Maccullochella peelii peelii* (Mitchell) (Percichthyidae) is a high priced freshwater fish which is gaining popularity as a suitable species for intensive culture (Ingram 2000; Ingram *et al.* 2004). Murray cod has been compared to the Chinese mandarin fish (*Siniperca chuasti*) which is considered to be the most valued warmwater freshwater species cultured commercially (De Silva 2001). Murray cod also has the added advantage of ease of weaning to dry feeds, unlike mandarin fish, which feeds only on live fish. Commercial culture of Murray cod is very recent, even though it has been artificially propagated in the past, primarily for stock enhancement purposes (Rowland 1983; Rowland 1988; Cadwallader and Gooley 1985). The biology of Murray cod also is well documented (eg. Harris and Rowland 1996), and Murray cod is known to be a top-order carnivore. Currently, Murray cod is cultured in intensive, indoor recirculating systems and in outdoor ponds. Being a new aquaculture species commercial diets formulated specifically for Murray cod are not yet available, and more often than not commercial farming operations use diets available for other cultured species, such as salmonids (particularly Atlantic salmon *Salmo salar*) and barramundi (*Lates calarifer*). Prior to the commencement of the present project there has been only one nutritional-related study of any form, in which it was reported that the "Blue-Sac" syndrome observed in artificially propagated Murray cod larvae could be caused by a deficiency of essential amino acids (EAA), over a long period of time, more commonly observed in old broodstock (Gunasekera *et al.* 1998).

The nutritional requirements of fish species differ widely, with carnivorous species generally tending to have relatively higher dietary protein requirements compared to omnivorous fish. Fish species also differ in their lipid dietary requirements, as well as the ability to utilise carbohydrates as an energy source (Wilson 1994). Feed costs typically account for 30-60% of the recurring cost of culturing carnivorous fish species, which are generally produced under intensive conditions in which all of the nutritional requirements of the

species are provided in the form of a complete, compound diet. Interestingly, Rawlinson (2004) has estimated feed to be about 29% of recurrent cost for Murray cod. As such, a pre-requisite for successful culture of intensively cultured species is an artificial diet that adequately and cost-effectively meets such requirements. However, to determine all the nutrient requirements (EAA, essential fatty acids (EFA), protein, lipid, energy, etc) of a new and developing aquaculture species is a long-drawn out process which generally requires a number of years of controlled experimentation, often at considerable cost. On the other hand, when time and funds are limited, and when the basic requirements of closely related species are known, nutritional investigations for candidate aquaculture species can be targeted on certain key areas that could be considered important for the development of suitable diet(s).

In the present study, this latter approach has been adopted with a view to "fast-tracking" the means of obtaining the minimum amount of useful nutritional information for formulating cost effective diets for Murray cod. Specifically the following experiments were conducted at Deakin University, Warrnambool, Victoria to:

- Determine the optimal protein requirement for juvenile Murray cod.
- Determine the most suitable dietary lipid level at pre-determined protein levels, and ascertain the protein sparing capabilities of juvenile Murray cod.
- Determine the digestibility of readily available products such as soybean meal (SBM), meat meal (MM) and shark meat meal (SMM) by Murray cod.
- Evaluate changes in amino acid and fatty acid composition in relation to development until yolk-sac resorption.
- Evaluate the possibilities of incorporating SBM in Murray cod diets, and other protein rich- ingredients such as blood meal (BM).
- Determine the possibilities of substituting the fish oil component with a crude oil preparation from trout offal.
- Conduct commercial scale trials based on diets formulated on the basis of all of the above experiments.

4.2 Materials and Methods

4.2.1 Facilities and experimental design

The basic design of the experimental systems used in the present study was similar throughout, with all experiments being carried out in various tank-based recirculating systems incorporating both continuous biological and solids filtration and supplementary aeration. Tank sizes for the different trials included 60 l, 160 l and 780 l capacity units, with the selection of tank size based on the initial size of the experimental animals. In all instances, each (dietary) treatment was carried out in triplicate under a 12:12 hr light:dark cycle and at a fixed temperature of 24 ± 1.0°C. Treatments were randomly assigned to tanks. During all experiments tanks were cleaned daily, and temperature, pH, NO_2 and NH_3 were monitored at least three times weekly using standard procedures (Helrich 1990). Fish were anaesthetised in MS 222 (1:10,000) when handled for weighing and measuring, and where necessary were euthanised in an overdose of MS 222.

4.2.2 Diet preparation and feeding

For diet preparation the ingredients (as required for each experiment) were thoroughly mixed, warm water added, then extruded through an appropriate sized die using a using a Heavy Duty Kitchen Aid (Kitchen Aid Inc., Michigan, USA). The resulting pellets were air dried until firm to the touch. The dry pellets were stored in batches at 4°C until used. Proximate composition analysis was carried out in triplicate on three samples of each experimental diet.

In general, fish were fed to apparent satiation each day, at approximately 0800-0900 hrs and 1500-1700 hrs, the point of satiation being considered when the fish showed no further interest in feeding. The amount of food offered to fish each day was recorded and used to determine daily feed consumption rates.

4.2.3 Growth and performance parameters

In all experiments that included an evaluation of test diets the following growth related criteria were estimated:

$$\text{Specific Growth Rate (SGR)} = \frac{(\ln Wt_2 - \ln Wt_1)}{(t_2 - t_1)} \times 100\%$$

$$\text{Food Conversion ratio (FCR)} = \frac{\text{Food consumed (g dry } Wt\text{) between } t_1 \text{ and } t_2}{\text{Increase in fish biomass (g wet } Wt\text{) between } t_1 \text{ and } t_2}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Increase in fish biomass (g wet } Wt\text{) between } t_1 \text{ and } t_2}{\text{Protein consumed (g dry } Wt\text{) between } t_1 \text{ and } t_2}$$

$$\text{Protein conversion efficiency (PCE)} = \frac{(\text{Body protein at } t_2 - \text{Body protein at } t_1)}{\text{Protein consumed (g dry } Wt\text{) between } t_1 \text{ and } t_2} \times 100\%$$

$$\text{Hepatosomatic Index (HSI)} = \frac{\text{Liver weight (g)}}{\text{Somatic weight (g)}} \times 100\%$$

$$\text{Net protein utilisation (NPU)} = \frac{\text{Increase in protein in carcass}}{\text{Total protein consumption}} \times 100\%$$

where: t_1 = Initial time.
t_2 = Final time.
Wt = Fish weight (g).
Wt_2 = Final fish weight (g) (at time t_2).
Wt_1 = Initial fish weight (g) (at time t_1).

4.2.4 Digestibility estimations

The protocol adopted for digestibility studies is given in detail in De Silva *et al.* (2000). In all the studies Cr_2O_3 was used as an external marker, and digestibility estimations were based on faecal samples accumulated through the night. In all instances the fish were acclimatised to the experimental diets for at least one week, prior to the collection of faecal samples for analysis, and during this period fish were gradually acclimated to the feeding, tank cleaning and faecal collection regimes that would be used during experimentation. The day before faeces were to be collected the tanks were thoroughly cleaned and siphoned following the afternoon feed to avoid contamination with residual food. Faeces were collected by siphoning, and frozen at -4°C before being freeze-dried. Faecal samples collected for each replicate of each treatment were pooled. The protocol adopted for faecal sample collection analysis, and subsequent estimations of apparent dry matter digestibility (ADMD) and nutrient digestibility has been previously described (De Silva *et al.* 2000). Freeze-dried faecal samples were analysed for chromic oxide (Cr_2O_3) (Furukawa and Tsukahara 1966), and protein and energy of faecal matter. ADMD, protein digestibility (PD)

and energy digestibility (ED) were calculated using standard formulae (Maynard and Loosli 1972; Cho and Slinger 1979). The formulae used were:

$$\text{Apparent dry matter digestibility } (ADC_{dm})\ (\%) = 100 - 100 \cdot \left[\frac{1 - (Cr_2O_3 \text{ in feed})}{(Cr_2O_3 \text{ in faeces})} \right]$$

$$\text{Nutrient digestibility } (\%) = 100 - 100 \cdot \left[\frac{1 - (Cr_2O_3 \text{ in feed})}{(Cr_2O_3 \text{ in faeces})} \cdot \times \cdot \frac{(\%\text{ nutrient in faeces})}{(\%\text{ nutrient in feed})} \right]$$

4.2.5 Chemical analyses

Samples of diets, ingredients and fish were sampled for chemical analysis. Whole carcass and/or a portion of the muscle, devoid of skin and bone, taken from the right fillet, were used for chemical analysis of fish. The muscle samples (a portion) and carcasses were oven dried at 80°C for 18 hrs.

4.2.5.1 Proximate analysis

Standard "Association of Official Analytical Chemists" (AOAC) procedures (Helrich 1990) were adopted in proximate analysis (protein by Kjeldahl nitrogen; total lipid by chloroform:methanol extraction; ash by burning in a muffle furnace at 550°C for 18 hrs; energy by burning in an oxygen atmosphere in a ballistic bomb calorimeter) of dietary ingredients, diets and carcass composition. Details of the methods used are given in De Silva *et al.* (2000).

4.2.5.2 Amino acid analysis

Sample preparation for total and free amino acid determinations were done according to Gunasekera *et al.* (1997; 1998). The samples were hydrolysed for 24 h at 100°C with 6 N HCl in sealed glass tubes replaced with nitrogen. An aliquot of an appropriate amount of the hydrolysate was taken, diluted with 0.25 M borate buffer, pH adjusted to 8.5, and was filtered through a 25 μm membrane filter. Free amino acids were segmented by homogenising (Ika-labortechnik homogenizer) in ice cold 6% trichloroacetic acid at a speed of 24,000 rpm, and centrifuging for 20 min at 8,400 rpm. The supernatant was freeze dried, dissolved in 0.25 M borate buffer, pH adjusted to 8.5, and filtered through a 25 μm membrane filter.

The pH adjusted samples were reacted with 9-fluroenylmethyl chloroformate (FMOC) to form amino acid FMOC derivatives using an automated GBC LC 1610 Autosampler, with a Hypersil column (150 mm L x 4.6 mm internal diameter). For both total and free amino acids, L-hydroxyproline was used as an internal standard and were analysed by pre column fluorescence derivative method using a fully automated, GBC LC 1150 HPLC (GBC Scientific Equipment, Australia). Resulting peaks were analysed using a Winchrom software package (GBC Scientific Equipment, Australia). All determinations were done in duplicate. Tryptophan was not estimated in this study.

4.2.5.3 Fatty acid analysis

The methods used for fatty acid analyses were the same as that used in our laboratory previously for studies on fatty acids (1997b; De Silva *et al.* 1997a). Briefly, sub samples were homogenised in chloroform-methanol (2:1, v/v) using a Ika-Labortechnik Ultra-Turrax T8 homogeniser and total lipid was extracted and estimated gravimetrically according to Folch *et al.* (1957). The fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5N methanolic NaOH and transesterified with 14% BF_3 (w/v) in methanol. Three aliquots of each of the three esterified samples (fatty acid methyl esters) were analysed in a Shimadzu GC 17A, equipped with an Omegawax 250 capillary column (30m L x 0.32 mm internal diameter), a FID detector and a split injection system (split ratio 50:1). The carrier gas was helium and injector port and detector temperatures were 240°C and 250°C, respectively. The temperature program was 190°C for 5 min, 190-240°C at 2°C min^{-1}, and held at 240°C for 10 min. Fatty acids were identified relative to known external standards and the resulting peaks were quantified using 23:0 as an internal standard (Sigma, USA).

The following lipid quality indices, developed by Ulbricht and Southgate (1991) were used to assess the lipid quality of fish. These were;

Atherogenicity Index (AI) = [12:0 + 4(14:0+16:0)] / [(n6+n3)PUFA+ 18:1+∑MUFA

Thrombogenicity Index (TI) = (14:0+16:0+18:0) / [(0.5 x 18.1) + 0.5 (∑ MUFA) + 0.5 (n6 PUFA) + 3 (n3 PUFA) + (n3 PUFA/ n6 PUFA)]

4.2.6 Statistical analysis

All data were first checked for homogeneity of variances (Zar 1984) using Levene's test. The data were then subjected to a one-way ANOVA, followed by Duncan's Multiple Range Test to determine significant differences amongst treatments, for growth parameters and proximate composition. All statistical analyses were performed using SPSS PC software package.

4.2.7 Experiments

4.2.7.1 Experiment 1: Determination of the optimal dietary protein requirement in juvenile Murray cod.

Dietary ingredients, fish meal (FM) (Peruvian origin), soybean meal (SBM) and vitamin and mineral pre-mixes used in the diets were purchased locally (Ridley Agriproducts, Brisbane, Queensland), and proximate composition analysis was carried out on three sub-samples (each in triplicate) of FM, SBM and wheat flour (WF). Based upon these analyses five isocalorific diets were formulated to contain 40%, 45%, 50%, 55% or 60% protein by dry weight and 10% lipid (Table 4.1). In the diet formulation it was ensured that the EFA and EAA contents were higher than those found in eggs and early larvae of Murray cod (Gunasekera *et al.* 1999a; 1999b), and were also above the limiting levels for carnivorous fish species (National Research Council 1993).

Fish (initial weight 21.5 g ± 0.3 s.e.) were distributed among 15 circular fibreglass tanks (18 fish per tank) of 160 l capacity. Temperature was 20±1.5°C, pH 7.2 and mean levels of ammonia and nitrite were below 0.1 ppm. Fish then acclimatised for one week before being weighed individually, in water, after anaesthetisation. Fish were fed to apparent satiation each day and were weighed in bulk at 14 day intervals. The experiment lasted 56 days. At the end of the experiment fish were anaesthetised, and individual weight determined.

In the course of the experiment, faecal collections were made every tenth day. On the days of faecal collection, the tanks were cleaned immediately following the morning feed, and at about 1400hrs, the faeces were carefully siphoned into a nylon mesh. Thus, the longest period faecal matter would have remained in the water was about 4 h. Faecal matter from dietary treatment replicates were pooled for each collection day, frozen, freeze-dried and kept in a desiccator until analysed.

At the start of the experiment, six fish were taken for proximate analysis and at the end six fish from each dietary treatment were sacrificed. Freshly killed fish were filleted, and the body muscle, devoid of skin, was stored frozen for proximate analysis.

4.2.7.2 Experiment 2: Comparison of digestibility and amino acid availability of three protein rich dietary ingredients.

Comparison of digestibility and amino acid availability was undertaken of three protein-rich dietary ingredients, SBM, SMM and MM (proximate composition given in Table 4.2) incorporated in pelleted compound diets. Specifically, standard digestibility experiments were carried out to determine the digestibility of these ingredients using a reference diet (RD) (Table 4.3) and test diets made up of 30% of test ingredient and 70% RD (De Silva *et al.* 2000). The ADM, PD and ED, and the amino acid availability ingredients were evaluated.

The proximate composition of the ingredients (M-% moisture, P-% protein, Tl-% total lipid, A-% ash and E-energy in kJ g-1) were; fish meal- 6.6M, 69.0P, 13.1Tl, 11.9A, 21.0 E; soybean meal- 7.3M, 46.0P, 3.3Tl, 6.8A, 19.1E; wheat flour- 8.6M, 10.8P, 0.7Tl, 0.6A, 19.0E.

Table 4.1. The ingredient (g kg diet-1) and proximate composition of the experimental diets used during Experiment 1. All values are on a dry weight basis.

Ingredients (g kg^{-1})	Diet code				
	P40	P45	P50	P55	P60
Protein level (%)	40	45	50	55	60
Fish meal	460	530	600	670	740
Soybean meal	120	140	160	180	200
Wheat flour	330	250	170	90	10
Vit. + Mineral Mix*	30	30	30	30	30
Oil**	40	30	20	10	----
CMC (Binder)	15	15	15	15	15
Cr_2O_3	5	5	5	5	5
Proximate Composition as is fed (analysed)					
% Moisture	6.4	6.3	6.7	6.3	6.0
% Protein	40.8	45.1	49.0	54.4	59.0
% Lipid	9.5	9.6	8.8	8.9	8.7
% Ash	8.2	9.3	8.7	10.5	12.7
Energy kJ g^{-1}	21.5	21.2	20.9	21.6	21.4
P/E (mg P kJ g^{-1})	52.7	47.0	42.7	39.7	36.3

* Commercial pre-mix from Ridley Agriproducts, Qld, Australia.

** 2:1 of cod liver oil: sunflower oil.

Table 4.2. The proximate composition of the test dietary ingredients (SBM- soybean meal (defatted); MM- Meat meal; and SMM- Shark meat meal), together with fish meal (FM; Chilean origin) and wheat flour (WF) used in the reference and test diets for Experiment 2. Percent protein, lipid, ash and energy are given for moisture free ingredient.

Parameter	Ingredient code				
	SBM	MM	SMM	FM	WF
Moisture (%)	4.8	0.1	83.2	6.6	0.6
Protein (%)	47.8	54.3	86.1	69.4	10.9
Lipid (%)	2.9	10.1	4.5	13.2	2.7
Ash (%)	7.0	29.4	6.3	11.9	0.6
Energy (kJ g^{-1})	19.1	18.0	26.1	21.0	18.6
Price per kg in A$**	0.52	0.38	-*	1.16	0.80

* Obtained free; waste product generally disposed at a cost of A$22mt.

** A$= O.63 US $; prices are for November 1998.

MM, SBM & FM were purchased from a feed manufacturer in Australia (Ridley Agriproducts Ltd., Brisbane).

Table 4.3. Ingredient (g kg -1 diet) and proximate composition (as fed basis) of reference diet (RD) used in the digestibility experiment (Experiment 2) on Murray cod.

Ingredient	Murray cod (RD-Mc)
Fish meal	700
Wheat flour	230
α- Cellulose	-
Vit+ Min. mix*	30
Oil (1:1 cod liver oil:sunflower oil)	15
Carboxy methyl cellulose (CMC)	15
Cr_2O_3	10
Proximate composition	
Moisture	1.4
Protein	50.9
Lipid	10.5
Ash	10.5
Energy (kJ g^{-1})	20.8

* Commercial Preparation (Ridley Agriproducts Ltd., Brisbane, Qld)

4.2.7.3 Experiment 3: Changes in amino acid and fatty composition of developing eggs and larvae, of Murray cod until yolk-sac resorption

Information on changes in the amino acids and fatty acids in eggs and larvae is thought to permit the estimation of nutritional requirements of exogenous feeding larvae (Tacon and Cowey 1985; Wilson and Poe 1985; Fraser *et al.* 1987). Such studies also provide a better understanding of utilisation of yolk reserves with early development. These aspects are important in optimising survival and improving larval quality in the artificial propagation of a species, and also provide information with regard to the potential variability amongst broodstock.

Murray cod spawn naturally in late spring and early summer at southern latitudes in Australia, in ponds provided with suitable spawning substrates for egg laying (Cadwallader and Gooley 1985). During this time artificial spawning substrates are checked for eggs regularly, and fertilised eggs are removed to the hatchery for incubation (20±1 °C), and subsequent larval rearing. A brief description of techniques used to collect, incubate and hatch eggs, and rear larvae is provided in Ingram and Larkin (2000). Since Murray cod spawned naturally in the ponds, it was not possible to obtain samples of pre-fertilised eggs for the present study.

Eggs were obtained from spawnings at the PIRVic, Eildon, Victoria. For each spawn, the diameter of a minimum of thirty fertilised eggs was determined under a dissecting microscope using an eye-piece micrometer. Briefly, total amino acid content of egg and larval samples were determined on two sub-samples each of about 20-30 eggs or larvae, from each spawn. Samples of eggs, newly hatched larvae (100-140) and yolk-sac resorbed larvae (100 to 140; prior to first feeding), from each spawn were collected, frozen and stored at -35°C for amino acid/fatty acid analysis. A separate set of samples (40-60 eggs and/or larvae) from each spawn was taken for proximate analysis. Each sample was divided into two sub-samples, which were treated identically and analysed separately in duplicate. As eggs and larvae from only those spawns which resulted in yolk-sac resorbed larvae were used the results of this study are based on four spawns of Murray cod.

4.2.7.4 Experiment 4: Performance of juvenile Murray cod fed diets with different protein to energy ratio (a study on protein sparing capabilities)

The dietary protein requirement of Murray cod, being a strict carnivore, is about 50% by dry weight, as indicated from the results of Experiment 1 of this study. A logical step in diet development for the species is to investigate its protein sparing capabilities through the use of dietary lipid as a major energy source. As such the present investigation was instigated to evaluate the response of juvenile Murray cod to diets of low (10%), medium (17%) and high (24%) lipid levels at dietary protein levels of 40% and 50% by dry weight. The present work complements previous work on the species on optimal dietary protein requirements (Experiment 1 this study, Gunasekera *et al.* 2000), digestibility studies on potential, protein-rich, dietary ingredients (Experiment 2 this study, De Silva *et al.* 2000), all of which are thought to contribute to the development of suitable diets for Murray cod.

The experiment was conducted on juvenile Murray cod with an initial mean weight of 15g, which were spawned and reared at PIRVic, Snobs Creek. Two series of diets, each of protein content of 40% and 50% (by dry weight) were formulated. The three 40% protein series diets had 10%, 17% and 24 % lipid, and the two 50% protein series diets had 17% and 24% lipid. These diets were designated the codes, $P_{40}L_{10}$, $P_{40}L_{17}$ and $P_{40}L_{24}$, and $P_{50}L_{17}$ and $P_{50}L_{24}$, respectively (Table 4.4). In order to obtain the desired protein and lipid levels in the 40% protein series diets, an inert substance, cellulose which is not known to be digested by fish (Stickney and Shumway 1974), was added. As the EAA and fatty acid requirements for Murray cod were not known at the time of diet formulation, it was ensured that the EAA requirements were above the limiting levels for that of carnivorous fish species (Wilson 1991; National Research Council 1993), and matched, as far as possible, to that of Murray cod eggs and larvae (Gunasekera *et al.* 1998; Gunasekera *et al.* 1999a; 1999b). Ingredient and proximate composition of the experimental diets are presented in Table 4.4. Fish were weighed in bulk at 14 day intervals, and at the end of the experiment were weighed individually. The experiment lasted 56 days.

The proximate composition of the ingredients (M- % moisture, P- % protein, Tl- % total lipid, A- % ash and E-energy in kJ g^{-1}) were; fish meal- 6.6M, 69.0P, 9.5Tl, 11.9A, 21.0 E; wheat flour- 0.6M, 10.8P, 2.6Tl, 0.6A,18.50E.

Table 4.4. Ingredient and proximate composition of the experimental diets for the protein sparing experiment (Experiment 4) on juvenile Murray cod.

Ingredients	Diet				
(g kg $^{-1}$)	P_{40} L_{10}	P_{40} L_{17}	P_{40} L_{24}	P_{50} L_{17}	P_{50} L_{24}
Fish meal	565	580	590	730	745
Wheat flour	225	150	80	140	55
α - Cellulose	130	120	110	---	--
Vit. + Min. Mix.**	30	30	30	30	30
Oil*	25	95	165	75	145
CMC (Binder)	25	25	25	25	25
Proximate composition (as fed basis)					
% Protein	40.9	41.3	40.5	51.1	50.0
% Lipid	9.9	16.8	24.6	16.6	23.8
% Ash	8.4	8.5	8.6	10.1	10.1
% NFE***	27.5	21.2	15.2	22.2	16.1
Energy kJ g^{-1}	16.4	18.2	19.8	20.3	21.8
P/E (mg P kJ g^{-1})	24.9	22.7	20.4	25.2	22.9

* 2:1 Cod liver oil : Sunflower oil.

** Commercial preparations (Ridley Agriproducts, Brisbane).

*** Nitrogen free extract (= 100 - % Moisture- % Protein- % Lipid- % Ash - % Fibre).

CMC Carboxy methyl cellulose.

4.2.7.5 Experiment 5: Growth and nutrient utilisation of Murray cod fingerlings fed diets with varying levels of soybean meal and blood meal.

This experiment was instigated to determine the use of SBM and BM in the diets of juvenile Murray cod. In this regard results of experiments on juvenile Murray cod fed diets with varying amounts of SBM and BM, as replacements for FM are presented. The experiment also included digestibility estimation of the experimental diets and an evaluation of the diets on carcass composition.

Juvenile Murray cod used in the experiments were spawned and weaned to a commercial salmon diet at the PIRVic, Snobs Creek (Ingram *et al.* 2001). Juvenile Murray cod with an initial weight of 2-3 g were transported to the Deakin University facilities and acclimatised in 760 L fibreglass tanks incorporated into a recirculating system (De Silva *et al.* 2000; Gunasekera *et al.* 2000). During the acclimatisation period the fish were fed a commercial diet (Kinta No. 1; Kinta Pyt. Ltd., Victoria, Australia) twice a day to apparent satiation.

Two series of isonitrogenous (50% protein as fed) and isoenergetic (20.2 kJ g^{-1}) diets were formulated, in each of which the FM protein was substituted by either SBM or BM protein in increments of 8%, up to 32%. The lipid level in the diets was maintained at 14%. In the control diet the FM was the only animal protein source. The ingredient composition and the proximate composition of the diets and ingredients are given in Table 4.5 and Table 4.6.

Juvenile Murray cod of similar size (3.2 ± 0.06 g) were randomly distributed into 35 L, circular fibreglass tanks, with a sloping bottom, incorporated into a recirculation system (in a constant temperature room) fitted with a biofilter and provided with constant aeration and a water flow of 4 L min^{-1} (De Silva *et al.* 2000; Gunasekera *et al.* 2000). Forty-five fish were introduced into each tank (total 27) and one of the nine experimental diets was randomly allocated to each. Fish were acclimatised for two weeks. During the experiment, which lasted for 70 days, fish were fed to apparent satiation twice a day. The experimental fish were bulk weighed in water at the end of the second, fifth and eight week. At termination (10th week) fish were weighed individually, and six fish from each tank were killed and frozen for carcass analysis at a later date.

Table 4.5. Ingredient and proximate (as fed) composition of the Experiment diets (Experiment 5). Diet designation indicates the % of fish meal (FM) substitution, either with soybean meal (SBM) or blood meal (BM). CD- control diet.

Ingredient	CD	Soybean meal diets				Blood meal diets			
g/Kg		8 SBM	16 SBM	24 SBM	32 SBM	8 BM	16 BM	24 BM	32 BM
Fish Meal	741	685	629	577	518	680	619	558	496
Soybean meal	0	83	165	247	329	0	0	0	0
Blood meal	0	0	0	0	0	47	94	141	188
Wheat flour	135	103	72	38	10	135	135	135	135
α- Cellulose	3	4	5	5	6	12	22	32	42
Vit + Min*	30	30	30	30	30	30	30	30	30
CMC	20	20	20	20	20	20	20	20	20
Oil**	66	70	74	78	82	71	75	79	84
Cr_2O_3	5	5	5	5	5	5	5	5	5
Proximate Composition (%)- Experimental Diets									
Moisture	6.1	9.4	7.6	8.8	4.8	9.4	8.8	5.2	5.1
Protein	49.9	48.1	49.3	49.3	50.7	48.7	48.0	49.5	50.1
Lipid	14.9	13.1	17.0	16.7	18.0	15.6	15.7	14.7	14.5
Ash	12.5	11.9	11.7	11.6	11.6	11.3	10.4	9.9	9.3
Energy (kJ/g)	20.0	20.3	20.5	20.7	20.8	20.8	20.5	20.1	20.7

* Commercial preparation, Ridley Agricultural Products Ltd., Australia.

** 2:1 cod liver oil: sunflower oil;

CMC carboxy methyl cellulose

Table 4.6. Proximate composition of the ingredients used in diets tested in Experiment 5

Parameter	Ingredient code			
	Fish meal	Soybean meal	Blood meal	Wheat flour
Moisture (%)	7.8	7.2	3.4	7.1
Protein (%)	65.6	48.7	85.5	10.4
Lipid (%)	9.4	2.8	3.0	3.2
Ash (%)	14.7	7.1	2.1	0.6
Energy (kJ g^{-1})	21.3	20.9	25.9	20.5

Faecal samples were collected during the experiment for digestibility estimations, after 14 days. Faeces were collected by siphoning in the morning before feeding, and frozen, freeze-dried and stored in a desiccator until analysed. Faecal samples were then collected every other day for 24 days. As the daily samples collected were not sufficient to perform all the analysis, those collected over three days, for each treatment, were pooled together. Feed and faecal samples from the fish fed the test diets were analysed for Cr_2O_3 (Furukawa and Tsukahara 1966) and protein, and the % ADMD and % PD of the diets determined.

4.2.7.6 Experiment 6: Evaluation of experimental diets under commercial scale

This experiment was instigated to determine whether the laboratory findings on nutritional aspects of Murray cod juveniles (previous experiments) can be utilised to develop a cost-effective diet, and to evaluate the influence of such a diet on fillet quality. The experiment was conducted over two trials. In the first trial (7 August - 8 November, 2000) the performance of fish reared on a laboratory formulated diet was compared with those grown on a commercially available salmon diet. In the second trial (12 September - 7 December, 2001), a similar comparison was carried out but in this instance defatted SBM in the laboratory formulated diet was increased significantly (to 20% of the protein content), and compared with a commercial diet used in barramundi culture. It should be noted that the commercial salmon and barramundi diets used in the experiment are both routinely used for the commercial production of Murray cod. The experiment also included an economic evaluation of the different diets.

The ingredient composition and the proximate composition of the laboratory formulated diets (Diets designated DU1 and DU2) used in the two trials, are given in Table 4.7. The laboratory formulated diets

were prepared in large quantities by a commercial feed manufacturer (Ridley Agri Products Pty. Ltd., Brisbane, Australia), based on the formulations provided by the present study. The commercial salmon diet (CD/ S) and barramundi diet (CD/ B), were manufactured and supplied by Skretting Australia, Tasmania, Australia (formerly Pivot Aquaculture). The proximate composition and the amino acid and fatty acid composition of all the diets used in the study are given in Table 4.7, Table 4.8 and Table 4.9, respectively.

Both trials were conducted at commercial fish farms. Trial 1 was undertaken at Australian Aquaculture Products Pty Ltd (AAP), Euroa, Victoria, Australia, while Trial 2 was undertaken at the Alexandra Fish Farm (AFF), Alexandra, Victoria, Australia. A more detailed description of these systems is provided in O'Sullivan (1999). During each trial, fish at both facilities were reared in 1,600 l circular plastic tanks (Plate 1e). Each tank received a continuous flow of water that was supersaturated with oxygen and maintained at a temperature of approximately 25°C and 20°C for Trial 1 and Trial 2, respectively. Duplicate tanks were used for each diet in each trial. Initial stocking densities for each tank were 109 kg m^{-3} and 72 kg m^{-3} for Trial 1 and Trial 2, respectively. At the beginning of each trial, every 3-9 weeks during each trial, and at the termination of each trial, random samples of 20-30 individual fish from each tank were anaesthetised and the weight and total length (TL) were taken. Fish were reared for 93 and 86 days in Trial 1 and Trial 2, respectively. Fish were fed daily to apparent satiation. Diets were fed into each tank over a continuous 24 hr period via automated belt feeders. Feed rates were presented as a percentage of dry weight of feed to wet weight of fish per day (% day^{-1}).

4.2.7.7 Experiment 7: Comparisons of the fillet quality of cultured and wild Murray cod

This experiment was instigated to compare the fillet quality (proximate and fatty acid composition) of cultured Murray cod, both non- purged and purged, and marketable size fish caught from the wild.

Fillet samples (centre portion of the right fillet) of wild Murray cod were obtained from surveys conducted by PIRVic staff. Samples were packed in ice and transported to the laboratory for chemical analysis (proximate and fatty acid composition). Wild fish, which were obtained from two sites (Ovens River, Wangaratta, Victoria and Goulburn River, Undera, Victoria) were 0.5–21 kg in weight and 34-100 cm in total length.

For comparison, marketable sized fish (600 g, 35 cm) cultured at AAP were obtained during the 2000 and 2001 harvests. Six non-purged fish from the 2000 harvest, and six non-purged and six purged fish from the 2001 harvest were used for analyses. In all instances the central portion of the right fillet were used and treated as indicated previously. Fish are purged for 5-7 days in running water at about 20° C. Murray cod used for the comparison with wild fish were reared on the commercially available barramundi diet (CD/B) during the last three months of the culture cycle.

4.3 Results

4.3.1 Experiment 1. Determination of the optimal dietary protein requirement in juvenile Murray cod

Overall food consumption (g) per fish per day for the different dietary treatments was 0.86±0.02 (P40), 0.81±0.02 (P45), 0.84±0.05 (P50), 0.84±0.02 (P55) and 0.94±0.04 (P60), which did not differ significantly between the treatments. The final mean weight, increase in mean weight (%) and SGR increased with increasing dietary protein up to P50 (Table 4.10), with growth being better ($P< 0.05$) in Murray cod maintained on diets P50, P55 and P60 than on P40 and P45.

Best FCR (1.05) and PER (1.98) were also observed in Murray cod reared on the P50 diet. The FCR of juvenile Murray cod reared on the P50 diet differed significantly from all the other treatments except fish on P55 diet (Table 4.10). The FCR (Y) to dietary protein content (X) relationship was best described by a second order polynomial curve (Fig. 4.1), and the mathematical relationship was:

$$Y = 0.0033X^2 - 0.346X + 10.196 \quad (r=0.95; P<0.01).$$

Based on the above equation the best estimated FCR occurs at a dietary protein level of approximately 53%. The best PER was also found in fish reared on the P50 diet, and was significantly higher than that for all the other diets (Table 4.10; Fig. 4.1). The HSI ranged from 1.79 (P55) to 2.63% (P40) and was significantly higher ($P< 0.05$) for the P40 and P45 diets than for other diets.

Table 4.7. The ingredient and proximate composition (mean + s.e.) of diets (as fed basis) used in the two commercial trials on Murray cod (Experiment 6). DU1 and DU2 are diets formulated in the present study, and CD/S and CD/B are commercial diets available for salmon and barramundi culture, respectively, in Australia.

Ingredient	Trial 1 (2000)		Trial 2 (2001)	
g kg^{-1}	DU1	CD/S*	DU2	CD/B*
Fish Meal[1]	650		580	
Defatted Soybean	95		200	
Wheat flour	100		75	
Oil[2]	105		90	
Vit + Min[3]	30		30	
CMC[4]	20			
Wheat gluten			25	
Wheat starch			75	
Proximate Composition - Experimental Diets				
Moisture (%)	3.5 ± 0.02	6.5 ± 0.02	6.9 ± 0.10	7.6 ± 0.09
Protein (%)	48.9 ± 0.07	46.6 ± 0.06	49.1 ± 0.15	44.4 ± 0.27
Lipid (%)	16.9 ± 0.06	21.7 ± 0.08	16.1 ± 0.27	19.5 ± 0.37
Ash (%)	11.2 ± 0.01	9.1 ± 0.05	10.4 ± 0.08	11.5 ± 0.02
Energy (kj g^{-1})	22.2 ± 0.25	22.9 ± 0.37	20.9 ± 0.06	21.0 ± 0.11

* Ingredient composition not available for commercial diets.

1 fish meal- Peruvian origin.

2 2:1 cod liver oil: sunflower oil.

3 Commercial vitamin and mineral preparation.

4 CMC carboxy methyl cellulose.

Note: DU1 and DU2 ingredients supplied by Ridley Agri Products Pty. Ltd., Brisbane.

Table 4.8. The mean (+ s.e.) amount of amino acids in the feeds (μmoles g feed-1; as fed basis) used in the trials (Experiment 6).

Amino acid	Trial 1 (2000)		Trial 2 (2001)	
	DU1	CD/S	DU2	CD/B
Arginine	116.5 ± 2.1	105.8 ± 1.1	122.7 ± 4.3	84.1 ± 1.4
Histidine	74.1 ± 0.4	60.8 ± 1.9	78.3 ± 3.1	50.9 ± 7.4
Isoleucine	72.9 ± 0.1	65.4 ± 1.2	74.9 ± 2.2	46.8 ± 1.3
Leucine	177.4 ± 4.3	183.7 ± 2.4	184.7 ± 8.8	139.2 ± 2.1
Lysine	424.2 ± 4.4	427.2 ± 17.9	514.9 ± 11.8	280.0 ± 28.3
Methionine	67.9 ± 1.0	59.9 ± 0.5	62.2 ± 2.0	43.7 ± 0.8
Phenylalanine	87.1 ± 0.3	85.8 ± 0.8	87.7 ± 2.9	64.1 ± 0.6
Threonine	128.1 ± 3.1	120.4 ± 1.8	130.4 ± 5.2	93.7 ± 3.4
Valine	92.9 ± 2.0	93.5 ± 1.7	93.8 ± 3.6	71.7 ± 1.3
Σ EAA	1241 ± 8	1202 ± 28	1350 ± 35	874 ± 24
Alanine	184.9 ± 8.3	183.1 ± 3.4	190.0 ± 14.8	148.5 ± 3.9
Aspartic acid	135.0 ± 4.6	115.7 ± 2.8	140.8 ± 14.6	86.3 ± 3.2
Cystine	33.3 ± 0.9	33.0 ± 0.6	31.8 ± 1.1	27.6 ± 4.1
Glutamic acid	197.5 ± 3.8	177.8 ± 3.9	206.6 ± 19.3	138.4 ± 4.5
Glycine	266.1 ± 3.4	257.3 ± 1.2	268.2 ± 7.9	238.4 ± 3.7
Proline	171.2 ± 0.9	176.6 ± 0.8	165.4 ± 3.3	158.2 ± 1.6
Serine	155.2 ± 4.2	167.1 ± 2.1	160.5 ± 7.3	140.8 ± 9.1
Tyrosine	59.0 ± 0.1	56.0 ± 0.6	61.6 ± 1.3	43.4 ± 1.0
Σ NEAA	1202 ± 24	1166 ± 14	1225 ± 69	981 ± 24
Σ TAA	2443 ± 32	2369 ± 41	2575 ± 104	1856 ± 18

Table 4.9. The mean (+ s.e.) amount of fatty acids in the feeds (μg mg feed-1; as fed basis) used in the trials (Experiment 6).

Fatty acid	Trial 1 (2000)		Trial 2 (2001)	
	DU1	CD/S	DU2	CD/B
14:0	5.9 ± 0.17	8.6 ± 0.25	6.6 ± 0.27	7.7 ± 0.12
16:0	28.2 ± 0.68	38.9 ± 0.81	30.2 ± 1.26	35.8 ± 0.63
18:0	4.5 ± 0.08	8.1 ± 0.11	6.4 ± 0.28	9.4 ± 0.19
20:0	0.4 ± 0.01	0.5 ± 0.01	0.5 ± 0.02	0.7 ± 0.08
22:0	0.2 ± 0.02	0.2 ± 0.01	0.2 ± 0.01	0.3 ± 0.02
Σ saturates	39.2 ± 0.94	56.3 ± 1.16	43.9 ± 1.86	53.9 ± 0.99
16:1n- 7	10.1 ± 0.24	15.4 ± 0.34	11.7 ± 0.45	13.7 ± 0.22
18:1n- 9	18.9 ± 0.38	25.1 ± 0.39	16.6 ± 0.74	23.9 ± 0.53
18:1n- 7	2.7 ± 0.05	4.3 ± 0.06	3.2 ± 0.14	4.1 ± 0.11
20:1n- 9	7.6 ± 0.12	1.8 ± 0.01	1.4 ± 0.05	1.7 ± 0.02
22:1n- 11	12.3 ± 0.28	0.4 ± 0.07	0.2 ± 0.01	0.4 ± 0.03
Σ monoenes	51.6 ± 0.80	47.1 ± 0.75	33.2 ± 1.41	43.8 ± 0.91
18:2n- 6	5.3 ± 0.12	5.3 ± 0.09	5.6 ± 0.31	6.6 ± 0.28
18:3n- 3	2.6 ± 0.04	1.9 ± 0.05	1.9 ± 0.09	2.0 ± 0.05
18:3n- 6	0.4 ± 0.00	0.9 ± 0.02	0.6 ± 0.08	0.8 ± 0.09
18:4n- 3	5.2 ± 0.10	5.6 ± 0.09	4.5 ± 0.24	5.3 ± 0.36
20:2n- 6	0.5 ± 0.03	0.3 ± 0.01	0.4 ± 0.04	0.5 ± 0.13
20:3n- 3	0.2 ± 0.01	0.2 ± 0.01	0.2 ± 0.01	0.3 ± 0.06
20:3n- 6	0.2 ± 0.01	0.3 ± 0.01	0.2 ± 0.04	0.2 ± 0.02
20:4n- 6	0.9 ± 0.01	1.7 ± 0.02	1.2 ± 0.06	1.4 ± 0.03
20:5n- 3	16.7 ± 0.30	30.6 ± 0.37	21.3 ± 0.79	24.0 ± 0.46
22:4n- 6	0.1 ± 0.01	0.2 ± 0.02	0.1 ± 0.0	0.1 ± 0.0
22:3n- 3	0.3 ± 0.00	0.4 ± 0.00	<0.10	<0.10
22:5n- 3	1.4 ± 0.02	2.6 ± 0.02	2.0 ± 0.08	2.6 ± 0.07
22:6n- 3	17.5 ± 0.30	18.6 ± 0.14	19.6 ± 0.85	18.9 ± 0.38
Σ n- 3	43.9 ± 0.78	59.9 ± 0.69	49.5 ± 2.05	53.1 ± 1.34
Σ n- 6	7.4 ± 0.15	8.8 ± 0.15	8.1 ± 0.45	9.6 ± 0.38
Σ PUFA	51.3 ± 0.94	68.6 ± 0.83	57.6 ± 2.49	62.7 ± 1.72
Σ HUFA	37.8 ± 0.66	54.9 ± 0.57	45.1 ± 1.77	48.1 ± 1.05
n-3/n-6	5.9 ± 0.03	6.8 ± 0.04	6.1 ± 0.12	5.6 ± 0.09

The ADMD, PD and ED ranged from 61.9 (P45) to 76.7% (P55), 86.4% (P45) to 91.5% (P55), and 62.8 to 77.9% (P50), respectively (Table 4.10). ED of the P50 diet was significantly higher than for P40, P45 and P60 diets. The PD did not differ significantly (P> 0.05) among diets, but there was a trend for the PD of diets P50 and P55 to be higher than for other diets.

The results of the proximate analysis of the body muscle, devoid of skin, are given in Table 4.11. Overall, there were no significant differences in the moisture, protein, total lipid and ash content in Murray cod fed diets of different protein content. However, all these parameters, except ash, in fish at the start of the study differed significantly (P< 0.05) from the corresponding values in fish on the different dietary treatments at the end of the experiment. It was however, noteworthy that the protein and lipid content, by dry weight, in Murray cod ranged from 91.3±0.19 (P55) to 92.8%±0.08 (P50), and 4.1±0.02 (P55) to 5.8%±0.04 (P60),

respectively. Based on the final carcass protein content the PCE in juvenile Murray cod ranged from 25.8 (P60) to 37.7% (P40), and decreased linearly with increasing dietary protein content; the relationship being:

Y = 62.76 – 0.62X (r= 0.99; P< 0.01), where X = dietary protein content and Y= PCE.

Table 4.10 Growth parameters (mean + s.e.) of Murray cod maintained under different dietary regimes, together with the results on digestibility of apparent dry matter (ADMD), protein (PD) and energy (ED) for the experimental diets (Experiment 1). Values with the same superscript are not significantly (P>0.05) different from each other.

Parameter	Dietary treatment				
	P40	P45	P50	P55	P60
Growth parameters					
Final weight (g)	52.0^{a} ±1.8	57.8^{ab} ±1.8	66.8^{c} ±2.7	60.9^{b} ±2.2	63.0^{bc} ±3.2
Increase in weight (%)	144.3^{a} ±10.6	161.7^{ab} ±8.9	206.3^{c} ±6.5	186.3^{bc} ±11.4	196.3^{c} ±8.9
SGR (% day^{-1})	1.59^{a} ±0.08	1.72^{ab} ±0.06	1.98^{d} ±0.04	1.87^{c} ±0.06	1.92^{cd} ±0.05
FCR	1.63^{c} ±0.11	1.27^{b} ±0.03	1.05^{a} ±0.04	1.2^{ab} ±0.14	1.23^{b} ±0.03
PER	1.51^{a} ±0.10	1.72^{b} ±0.03	1.98^{c} ±0.11	1.57^{ab} ±0.17	1.37^{a} ±0.03
HSI (%)	2.63^{b} ±0.24	2.31^{b} ±0.15	1.95^{a} ±0.08	1.79^{a} ±0.13	1.87^{a} ±0.13
Digestibility (%)					
ADMD	69.6^{ab} ±3.7	61.9^{a} ±1.6	74.5^{bc} ±0.3	76.7^{bc} ±3.1	71.1^{b} ±2.6
PD	87.8 ±1.8	86.4 ±2.4	90.6 ±0.5	91.5 ±1.2	88.0 ±1.6
ED	64.0^{ab} ±3.9	62.8^{a} ±1.5	77.9^{c} ±0.8	75.9^{bc} ±3.1	73.5^{b} ±2.8

Table 4.11. Proximate analysis (mean + s.e.) of the body muscle of Murray cod at the commencement of the study (initial) and for different dietary treatments (Experiment 1). Values with the same superscript are not significantly (P> 0.05) different from each other.

Parameter	Dietary treatment					
(% wet weight)	Initial	P40	P45	P50	P55	P60
Moisture	64.8±1.84^{a}	73.0±0.13^{b}	72.5±0.14^{b}	72.9±0.34^{b}	73.3±0.19^{b}	73.6±0.85^{b}
Protein	31.5±3.13^{b}	24.8±0.1^{a}	25.2±0.23^{a}	25.2±0.33^{a}	24.4±0.22^{a}	24.1±0.82^{a}
Total lipid	1.72±0.06^{b}	1.13±0.02^{a}	1.13±0.03^{a}	1.11±0.01^{a}	1.07±0.00^{a}	1.52±0.02^{a}
Ash	1.99±0.19	1.57±0.02	1.81±0.13	1.71±0.04	1.69±0.17	1.66±0.03

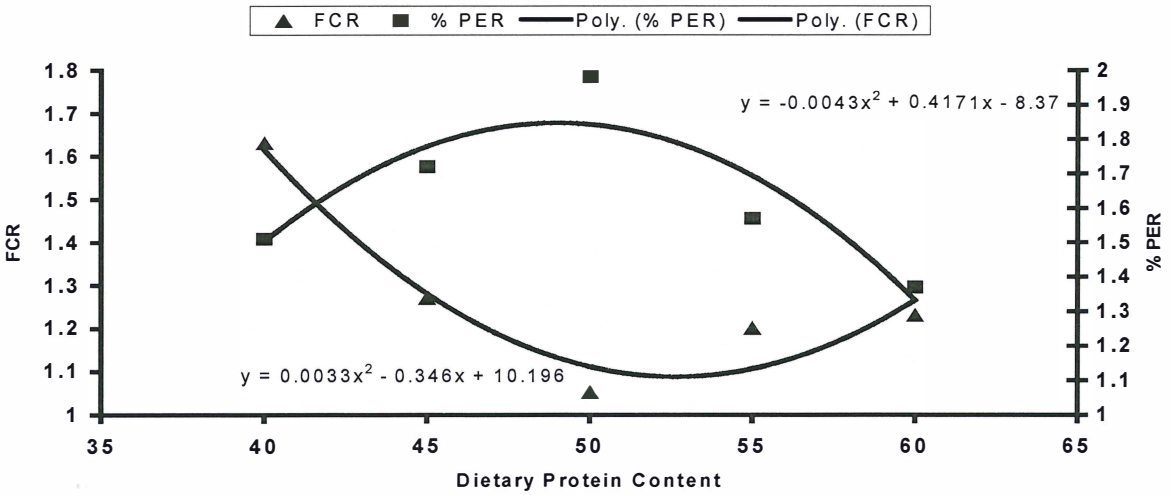

Fig. 4.1. Relationship of % dietary protein content to FCR and % PER (Experiment 1).

4.3.2 Experiment 2. Comparison of digestibility and amino acid availability of three protein rich dietary ingredients

4.3.2.1 ADMD and nutrient digestibility

The mean percent (± s.e.) ADMD, PD and ED of reference and test diets, together with those of the test ingredients, for Murray cod are summarised in Table 4.12. The highest ADM of the test diets was observed for the SMM (73.1 ± 1.58) incorporated diets in Murray cod and were significantly higher than that for the other test diets.

The highest PD was also observed for SMM (87.5 ± 1.27) incorporated diets in Murray cod (Table 4.12). However, unlike in the case of ADMD, the differences in PD amongst diets were not always significant. In Murray cod the PD of MM incorporated diets was the lowest, and significantly so than all the experimental diets in the latter. The ED of the diets did not differ significantly in from each other (note that ED could not be estimated for SMM substituted diets due to lack of samples).

As for ingredient digestibility in Murray cod, ADMD (104.8 ± 7.28), PD (100.9 ± 04.40) and ED (88.8 ± 5.4) of SMM were higher than that for other ingredients, not always significantly, however (Table 4.12). In Murray cod, PD of SBM was better than that of MM ($p<0.05$). The lowest ED was observed for SBM, significantly lower than SMM and MM in the case of Murray cod.

Table 4.12. The mean (+ s.e.) percent apparent dry matter digestibility (ADMD), protein digestibility (PD) and energy digestibility (ED) of reference diet and test diets used in the digestibility experiments on Murray cod. Values with the same superscript are not significantly different (P>0.05).

Digest	Diet			
	Reference Diet (RD)	Soybean meal (SBM)	Shark meat meal (SMM)	Meat meal (MM)
Percent Digestibility of different diets				
ADM	59.4^{a} ±1.71	65.5^{b} ±1.56	73.1^{c} ±1.58	64.6^{ab} ±2.41
PD	80.4^{a} ±1.23	83.6^{ab} ±1.31	87.5^{b} ±1.27	78.3^{a} ±1.92
ED*	73.1 ±2.81	68.6 ±3.32	77.8 ±3.20	72.1 ±2.61
Ingredient digestibility				
ADM		79.5^{a} ±5.21	104.8^{b} ±7.28	82.7^{ab} ±7.86
PD		93.9^{ab} ±4.10	100.9^{b} ±4.40	81.3^{a} ±5.70
ED		58.3^{a} ±8.30	88.8^{b} ±5.4	70.0^{b} ±6.70

* insufficient samples available for analysis.

4.3.2.2 Amino acid availability of test diets and ingredients

The amino acid composition of the three test ingredients (SBM, SMM and MM), together with FM and that of Murray cod is given in Table 4.13. Total EAA availability, total NEAA availability and TAA availability for SBM and SMM incorporated diets were high. The availability of individual amino acids, EAA availability, NEAA availability and TAA availability were estimated for the reference diets and test diets for Murray cod (Table 4.14). In Murray cod TEAA availability, NEAA availability and TAA availability (89.7, 82.1 and 85.2%, respectively) were significantly higher (except for total NEAA availability) for the MM incorporated diet than for the reference diet (84.5, 77.6 and 80.6%, respectively), and all EAA of this diet were available in excess of 82% (Table 4.14). The availability of individual EAA availability in the different diets, differed significantly in respect of some EAA. In Murray cod, no clear trend was apparent in respect of individual EAA availability. However, significant differences were evident in respect of all EAA, except leucine and threonine amongst the different diets.

Table 4.13. The amino acid composition in μ moles per g-1 of test ingredients, on a dry weight basis and that of Murray cod (mean of two size groups) on a wet weight basis. For comparison that of the fish meal used in the present study is also given.

Amino acid	Soybean meal (SBM)	Shark meat meal (SMM)	Meat meal (MM)	Fish meal (FM)	Murray cod
Essential (EAA)					
Arginine	111.3 ± 3.9	162.5 ±11.0	123.9 ±2.5	134.3 ±2.4	48.7 ±1.3
Histidine	53.7 ± 1.7	53.9 ±6.6	48.7 ±1.2	91.7 ±3.8	22.6 ±0.7
Isoleucine	41.7 ± 1.7	57.8 ±3.2	27.6 ±0.5	53.3 ±0.9	34.1 ±0.8
Leucine	177.1 ± 6.4	192.2 ±12.4	185.7 ±2.9	213.0 ±2.6	80.8 ±2.0
Lysine	141.1 ± 2.3	194.0 ±13.2	158.1 ±7.2	199.1 ±4.9	96.8 ±1.9
Methionine	50.6 ± 2.9	112.3 ±8.3	60.3 ±1.3	107.1 ±1.8	29.4 ±1.4
Phenylalanine	84.4 ± 2.9	93.9 ±4.5	77.0 ±1.3	87.9 ±1.6	34.7 ±0.9
Threonine	120.8 ± 4.9	206.1 ±13.0	226.7 ±18.3	178.9 ±3.4	55.2 ±1.2
Valine	55.3 ± 2.0	69.4 ±4.9	69.9 ±1.5	75.6 ±1.2	40.9 ±1.0
∑EAA	819	1142	974	1141	443
Non- essential (NEAA)					
Alanine	145.9 ±8.3	236.9 ±19.0	277.0 ±12.5	226.1 ±6.1	86.9 ±1.4
Aspartic acid	205.0 ±12.1	185.8 ±9.6	158.3 ±8.0	240.4 ±7.7	86.2 ±2.5
Cystine	104.3 ±3.8	97.9 ±7.5	75.5 ±0.8	119.0 ±5.4	24.5 ±0.7
Glutamic acid	322.6 ±6.6	235.0 ±15.3	226.1 ±9.9	285.1 ±6.5	122.6 ±3.2
Glycine	187.6 ±2.6	582.6 ±23.4	505.6 ±20.1	317.4 ±4.4	97.3 ±1.6
Proline	164.2 ±2.8	267.9 ±9.6	299.9 ±3.2	219.7 ±3.1	56.4 ±0.6
Serine	167.7 ±6.2	184.7 ±11.6	153.5 ±3.9	188.5 ±3.2	63.6 ±1.2
Tyrosine	59.6 ±2.0	70.4 ±3.5	51.8 ±0.7	74.2 ±2.1	38.6 ±1.4
∑NEAA	1342	1861	1748	1670	576
TAA	2161	3003	2722	2811	1019

Table 4.14. Availability of individual EAA, total EAA, total NEAA and TAA in Murray cod for the reference diet (RD) and test diets incorporated with 30% of the test ingredients. For each amino acid or group of amino acid, values with the same superscript are not significantly different (P>0.05)

Amino acid	Reference diet (RD)	Soybean meal (SBM)	Shark meat meal (SMM)	Meat meal (MM)
Arginine	82.7^{a} ±1.31	91.2^{b} ±1.91	87.2^{ab} ±1.49	84.4^{a} ±0.53
Histidine	91.7^{ab} ±0.46	90.1^{a} ±0.84	93.1^{bc} ±0.77	94.5^{c} ±0.51
Isoleucine	84.9^{a} ±1.96	88.9^{ab} ±0.74	87.6^{ab} ±1.43	90.4^{ab} ±0.73
Leucine	86.0^{ab} ±1.19	84.5^{a} ±1.11	88.1^{b} ±1.41	90.2^{b} ±0.63
Lysine	82.2^{ab} ±2.96	75.8^{a} ±4.61	78.5^{a} ±3.01	93.9^{b} ±1.42
Methionine	83.0^{a} ±1.87	90.0^{ba} ±1.96	84.7^{ab} ±2.62	89.2^{b} ±1.19
Phenylal.	84.9^{ab} ±1.92	83.5^{a} ±1.24	87.4^{ab} ±1.54	89.2^{b} ±1.13
Threonine	82.4 ±2.24	88.4 ±1.14	86.5 ±3.31	84.9 ±2.50
Valine	84.9^{a} ±1.09	84.2^{a} ±1.12	86.8^{ab} ±1.47	89.8^{b} ±0.63
EAA	84.5^{a} ±1.42	85.7^{ab} ±0.94	86.5^{ab} ±1.80	89.7^{b} ±0.95
NEAA	77.6^{a} ±1.57	83.2^{b} ±1.26	82.3^{ab} ±1.70	82.1^{ab} ±0.96
TAA	80.6^{a} ±1.50	84.2^{ab} ±1.11	83.9^{ab} ±1.74	85.2^{b} ±0.87

4.3.3 Experiment 3. Changes in amino acid and fatty acid composition of developing eggs and larvae, of Murray cod until yolk-sac resorption

The present study was conducted on samples obtained from four spawns of Murray cod. The total length and weight of Murray cod females of which spawns were used in this study ranged from 59.0 to 82.0 cm and 3.0 to 8.32 kg, respectively. The percentage of fertilisation and hatching exceeded 60% for all the spawns used in the present study. The average number of days taken from fertilisation to hatching, and hatching to

yolk-sac resorption were 8-9 and 7-8 days, respectively (at 20±1.5°C). The mean diameter of fertilised eggs was 3.52±0.09 mm.

4.3.3.1 Changes in amino acid composition

The mean amount of individual total EAA, and total NEAA in the TAA pool (protein bound + free) for each stage of development in Murray cod are given in Table 4.15. Nine EAA and eight NEAA were quantified in the TAA pool at all stages of development. With the exception of aspartic acid and glycine, all the others decreased with development. The amount of individual amino acids present in the different developmental stages was not always significantly different from each other (Table 4.15). For example, the amount of arginine, histidine, phenylalanine and threonine in yolk-sac resorbed larvae were lower ($P<0.05$) than in all the other developmental stages, but the differences amongst unfertilised eggs, fertilised eggs and newly hatched larvae were not.

The changes in the individual free essential amino acids (FEAA), and non-essential free amino acids (FNEAA) in the free amino acid (FAA) pool with development in Murray cod are given in Table 4.15. During ontogeny from fertilised egg to yolk-sac resorbed larva the FAA content increased ($P<0.05$) from 36 to 217 n mol ind^{-1} (Table 4.15). This trend was reflected in both FEAA and FNEAA of the FAA pool. In the FAA pool leucine and lysine were found in highest quantities in fertilised eggs, but in yolk-sac resorbed larvae threonine was the highest (Table 4.15). Of the FNEAA alanine was the highest in all the development stages investigated. Unlike in the case of individual amino acids in the TAA pool, increases in FEAA and FNEAA of the FAA pool in the transformation from fertilised egg to newly hatched larva and from newly hatched larva to yolk-sac resorbed larva were significant ($P<0.05$), except in the case of methionine. The FAA pool was only a small proportion of the TAA pool. The percentage of FAA in the TAA pool ranged from 0.19 to 1.64% in fertilised eggs and yolk-sac resorbed larvae and a significant increase was evident from egg to newly hatched larva only.

Table 4.15. Total (protein bound + free) and free amino acid content (+ s.e.) in μ moles per egg or larva and n moles, respectively of fertilised eggs (FE), newly hatched larvae (NHL) and yolk-sac resorbed larvae (YSRL) of Murray cod. Values with the same superscript in each row are not significantly different (P>0.05).

Amino acid	Total amino acid (μ moles)			Free amino acids (n moles)		
	FE	NHL	YSRL	FE	NHL	YSRL
Essential amino acids (Protein bound + free)						
Arginine	1.0±0.3^{b}	0.8±0.1^{b}	0.7±0.0^{a}	2.5±0.4^{a}	12.3±1.5^{b}	11.8±1.5^{b}
Histidine	0.5±0.0^{b}	0.4±0.0^{a}	0.3±0.0^{a}	1.8±0.3^{a}	7.3±1.0^{b}	16.3±1.3^{c}
Isoleucine	0.9±0.0^{b}	0.8±0.1^{b}	0.6±0.0^{a}	1.7±0.3^{a}	14.2±1.8^{b}	14.3±1.7^{b}
Leucine	1.9±0.1^{b}	1.7±0.1^{b}	1.3±0.1^{a}	3.4±0.4^{a}	19.0±2.5^{b}	15.9±1.5^{b}
Lysine	1.3±0.1^{b}	1.1±0.1^{a}	0.9±0.1^{a}	3.4±0.5^{a}	14.0±1.9^{b}	14.9±1.1^{b}
Methionine	0.7±0.0^{c}	0.6±0.0^{b}	0.5±0.0^{a}	1.2±0.2^{a}	10.1±1.2^{c}	7.5±0.9^{b}
Phenylal.	0.6±0.0^{b}	0.5±0.0^{a}	0.5±0.0^{a}	1.1±0.2^{a}	7.9±1.0^{b}	8.2±1.0^{b}
Threonine	1.2±0.0^{c}	1.0±0.1^{b}	0.8±0.1^{a}	1.5±0.2^{a}	11.0±1.9^{b}	18.2±1.7^{c}
Valine	1.0±0.0^{c}	0.8±0.1^{b}	0.6±0.0^{a}	2.6±0.5^{a}	12.1±1.7^{b}	13.7±1.5^{b}
Σ TEAA	9.0±0.3^{c}	7.7±0.5^{b}	6.1±0.4^{a}	19.3±2.9^{a}	107.9±14.2^{b}	120.8±11.5^{b}
Non-essential amino acids (Protein bound + free)						
Alanine	2.2±0.1^{b}	2.0±0.1^{b}	1.5±0.1^{a}	5.7±0.8^{a}	25.8±3.5^{b}	40.1±3.1^{c}
Aspartic	1.1±0.1^{a}	0.9±0.1^{a}	0.9±0.1^{a}	1.2±0.2^{a}	3.5±0.3^{b}	5.2±0.5^{c}
Cystine	0.6±0.0^{b}	0.6±0.0^{b}	0.4±0.0^{a}	tr	tr	tr
Glutamic	1.6±0.1^{b}	1.2±0.1^{a}	1.3±0.1^{a}	2.6±0.3^{a}	5.3±0.8^{b}	11.0±1.1^{c}
Glycine	1.0±0.0^{a}	1.0±0.0^{a}	1.1±0.0^{a}	1.8±0.4^{a}	8.1±1.3^{b}	9.5±0.9^{b}
Proline	1.3±0.1^{b}	0.9±0.1^{a}	0.8±0.0^{a}	0.7±0.1^{a}	2.5±0.5^{b}	2.7±0.3^{b}
Serine	1.5±0.0^{b}	1.3±0.1^{b}	1.1±0.1^{a}	3.4±0.6^{a}	13.3±1.7^{b}	17.3±1.7^{b}
Tyrosine	0.7±0.0^{c}	0.5±0.0^{b}	0.4±0.0^{a}	1.7±0.4^{a}	10.4±1.5^{b}	10.8±1.4^{b}
Σ TNEAA	10.0±0.2^{b}	8.5±0.5^{a}	7.6±0.4^{a}	16.9±2.5^{a}	68.9±9.2^{b}	96.6±7.4^{c}
Σ TAA	19.0±0.5^{c}	16.2±1.0^{b}	13.7±0.8^{a}	36.2±5.3^{a}	176.8±23.^{b}	217.4±18.^{b}
FAA/TAA (%)				0.19^{a}	1.18^{b}	1.64^{b}

4.3.3.2 Changes in fatty acid composition

In Murray cod the fatty acids accounted for more than 80% of the total lipid in all the developmental stages investigated. However, the percentage of fatty acids in total lipid in newly hatched larvae was lower ($p<0.05$) than in the other two developmental stages investigated. In all the developmental stages studied, PUFA accounted for more than 50% of the fatty acids in total lipid, and the contribution of n-3 and n-6 series fatty acids to the pool was higher than saturates and/or monoenes. Also, the percent changes in saturates, monoenes and PUFA (n-3 and n-6) in total fatty acid pool with development were not significant. The n-3 to n-6 ratio in Murray cod during ontogeny ranged between 1.07-1.19.

In Murray cod, 19 fatty acids were identified (Table 4.16) and quantified for all the developmental stages investigated. In addition, three other fatty acids [20:4(n-3), 18:3(n-6), and 22:5(n-6)] occurred in trace amounts only. The changes in individual fatty acids in total lipid, expressed in μg per mg total lipid, are given in Table 4.16. The fatty acids that occurred in highest abundance, through all the developmental stages, in order were docosahexaenoic acid [DHA; 22:6(n-3)], arachidonic acid [AA; 20:4(n-6)], oleic acid [18:1(n-9)] and palmitic acid (16:0), all of which exceeded 100 μg mg total lipid^{-1} in most instances (Table 4.16). During ontogeny, from egg to yolk-sac resorbed larva only, one fatty acid (16:1n-7) decreased significantly. In contrast, the amount of 20:4(n-6) in total lipid increased ($p<0.05$) during ontogeny. In general, the same trends were evident as previously indicated, except in a few instances when the differences were more pronounced. For example, the only PUFA that decreased during embryogenesis were 18:2(n-6) and 18:3(n-3) (Table 4.16).

Table 4.16. The mean amount of individual fatty acids in μg per mg total lipid (+ s.e.) in eggs and/or larva of Murray cod during development. Individual fatty acids with the same superscript are not significantly different ($p>0.05$) between developmental stages. FE - fertilised eggs; NHL - newly hatched larvae and YSRL - yolk-sac resorbed larvae. 18:3(n-6), 20:4(n-3) and 22:5 n-6 were found in trace amount. 16:1(n-9) and 20:1(n-9) were not detected.

Fatty acid	In μg per mg Total lipid (± s.e.)			In μg per egg and/or larva		
	FE	NHL	YSRL	FE	NHL	YSRL
14:0	3.9 ±1.2	2.4 ±0.2	4.3 ±0.3	4.1 ±1.3	2.9 ±0.4	3.6 ±0.3
16:0	102.3 ±3.0	98.4 ±2.2	104.3 ±3.2	100.9^{ab} ±4.4	113.2^{b} ±7.7	90.1^{a} ±6.0
18:0	70.4 ±2.4	69.2 ±2.4	74.2 ±2.4	69.1^{a} ±1.8	78.5^{b} ±2.1	63.8^{a} ±3.4
20:0	0.8^{ab} ±0.0	0.6^{a} ±0.1	1.2^{b} ±0.1	0.8 ±0.0	0.7 ±0.1	1.0 ±0.0
Σ saturates	177.7 ±4.6	170.9 ±3.8	184.1 ±5.4	175.0^{ab} ±6.5	195.4^{b} ±10.1	158.6^{a} ±9.4
16:1n-7	32.9^{b} ±2.9	27.2^{ab} ±1.8	24.9^{a} ±1.6	32.2^{b} ±3.3	31.6^{b} ±3.4	21.8^{a} ±2.3
18:1n-9	117.5 ±2.5	114.4 ±1.4	113.6 ±2.5	115.7^{b} ±3.8	131.1^{b} ±6.9	98.3^{a} ±6.4
18:1n-7	47.0 ±2.8	43.6 ±1.4	41.8 ±1.4	46.4^{ab} ±3.5	50.3^{b} ±3.9	36.4^{a} ±2.8
20:1n-9	3.7^{b} ±0.2	2.7^{a} ±0.2	3.6^{b} ±0.2	3.6^{b} ±0.2	3.0^{a} ±0.1	3.0^{a} ±0.1
Σ monoenes	193.1 ±7.8	188.0 ±3.5	184.1 ±5.3	198.4^{b} ±9.5	216.0^{b} ±13.7	159.7^{a} ±11.4
18:2n-6	63.6 ±4.7	55.1 ±4.4	53.4 ±0.8	63.1^{b} ±5.9	64.1^{b} ±8.0	46.4^{a} ±3.2
18:3n-3	11.0 ±3.4	9.0 ±2.5	7.6 ±1.6	11.2^{b} ±3.7	11.2^{b} ±3.8	6.9^{a} ±1.8
18:4n-3	3.7 ±0.3	3.0 ±0.4	2.8 ±0.4	3.6^{b} ±0.3	3.3^{ab} ±0.4	2.4^{a} ±0.3
20:2n-6	4.1 ±0.8	3.5 ±0.5	3.7 ±0.5	4.1 ±0.9	4.2 ±0.8	3.3 ±0.6
20:3n-3	28.3^{ab} ±1.7	35.2^{b} ±3.1	26.5^{a} ±3.0	28.2^{a} ±2.3	41.0^{b} ±4.8	24.0^{a} ±3.7
20:3n-6	17.9 ±2.8	14.2 ±2.1	17.4 ±2.8	17.2 ±2.5	15.4 ±1.9	14.6 ±2.5
20:4n-6	118.2^{a} ±7.3	110.2^{a} ±9.4	154.1^{b} ±12.1	115.8 ±6.4	124.8 ±9.7	129.6 ±6.0
20:5n-3	19.8 ±2.9	18.8 ±2.8	21.1 ±1.5	19.7 ±3.3	22.1 ±4.9	18.3 ±1.9
22:4n-6	16.5^{ab} ±0.9	13.5^{a} ±1.1	19.8^{b} ±1.9	16.1 ±0.6	15.0 ±0.5	16.6 ±1.4
22:5n-3	48.7 ±5.6	38.0 ±4.2	43.5 ±5.4	47.2 ±4.8	44.2 ±3.0	36.7 ±4.5
22:6n-3	143.8 ±10.9	130.6 ±11.8	164.2 ±10.4	141.9 ±12.5	150.2 ±17.1	139.3 ±6.6
Σ n-3	255.4 ±18.3	234.7 ±17.2	265.9 ±13.3	252.0 ±21.1	270.3 ±27.7	227.7 ±13.6
Σ n-6	220.5^{ab} ±11.2	197.0^{a} ±14.2	248.5^{b} ±15.7	216.5 ±10.8	224.0 ±16.7	210.7 ±10.3
Σ PUFA	476.0 ±28.7	431.8 ±30.6	514.5 ±27.7	468.6 ±31.6	494.4 ±44.0	438.5 ±22.4

4.3.4 Experiment 4: Performance of juvenile Murray cod fed diets with different protein to energy ratio (a study on protein sparing capabilities)

The performance indicators of juvenile Murray cod for each of the treatments are given in Table 4.17. The SGR ranged from 1.18 to 1.41 % day^{-1}, and was not significantly different between dietary treatments, except $P_{40}L_{10}$ and the rest. However, there was a general tendency for SGR to increase with increasing dietary lipid content at both protein levels. The trends in FCR and PER for the dietary treatments broadly reflected those for SGR. The FCR for the 40 % protein series diets were poorer compared to those of the 50% protein diets, and the best FCR of 1.14 was observed with the $P_{50}L_{17}$ diet. On the other hand, the PER was better in fish reared on the low protein diets. The net protein utilisation (NPU) also did not differ significantly ($P > 0.05$) in relation to dietary treatment. However, as in the case of PER the highest NPU was observed in Murray cod reared on diet $P_{40}L_{24}$ and the lowest in those on diet $P_{50}L_{24}$. The HSI tended to be higher with the low and medium levels of dietary lipid than for the other diets. All in all, the differences between parameters were not always significant between treatments. The carcass lipid content reflected that of the diets (Table 4.18), when significant increases in the lipid content was observed in relation to dietary lipid content at both protein levels. In all instances, the carcass lipid content was significantly higher ($P < 0.05$) than the initial. However, the lipid content of body muscle was much lower (Table 4.19), and did not change in relation to the dietary lipid content. The carcass moisture and protein did not change significantly in relation to the dietary treatments.

Table 4.17. Mean (+ s.e.) final body weight, Food Consumption (FC), Percent Specific Growth Rate (% SGR), Food Conversion Ratio (FCR), Protein Efficiency Ratio (PER), Net protein utilisation (NPU) and Hepatosomatic Index (HSI) in juvenile Murray cod in relation to different dietary treatments. For any one parameter values with the same superscript are not significantly different (P> 0.05).

Parameter	Dietary treatment				
	P_{40} L_{10}	P_{40} L_{17}	P_{40} L_{24}	P_{50} L_{17}	P_{50} L_{24}
Final weight (g)	29.0 ^{a} ±0.60	31.5 $^{a\,b}$ ±0.30	32.2 $^{a\,b}$ ±0.60	33.1 $^{a\,b}$ ±0.10	33.3 ^{b} ±1.20
FC (mg fish^{-1} day^{-1})	252 ±24.4	251 ±24.6	254 ±24.8	278 ±26.6	289 ±29.2
SGR (% day^{-1})	1.18 ^{a} ±0.02	1.31 ^{b} ±0.02	1.36 ^{b} ±0.03	1.37 ^{b} ±0.01	1.41 ^{b} ±0.04
FCR	1.40 ±0.05	1.22 ±0.12	1.20 ±0.11	1.14 ±0.04	1.18 ±0.07
PER	1.77 ±0.06	2.03 ±0.06	2.08 ±0.23	1.75 ±0.06	1.69 ±0.10
HSI	2.66 ^{b} ±0.19	2.23 $^{a\,b}$ ±0.21	1.68 ^{a} ±0.05	1.97 ^{a} ±0.15	1.95 ^{a} ±0.19
NPU (%)	28.3 ±1.00	31.7 ±0.95	31.8 ±3.50	28.0 ±1.09	26.3 ±1.67

Table 4.18. The whole body proximate composition (+ s.e.) of juvenile Murray cod at the commencement of the experiment and at termination in relation to different dietary treatments. Each analysis is based on six fish from each replicate. In any row values with the same superscript are not significantly (P> 0.05) different.

Parameter	Initial	Dietary treatment				
		P_{40} L_{10}	P_{40} L_{17}	P_{40} L_{24}	P_{50} L_{17}	P_{50} L_{24}
Moisture	77.4 ^{b} ±0.70	75.7 ^{a} ±0.34	75.0 ^{a} ±0.47	74.4 ^{a} ±0.78	75.3 ^{a} ±0.30	74.2 ^{a} ±0.18
Protein	15.1 ±0.05	15.4 ±0.03	15.3 ±0.15	15.2 ±0.01	15.5 ±0.23	15.3 ±0.06
Lipid	4.0 ^{a} ±0.01	5.4 ^{b} ±0.14	6.3 ^{c} ±0.26	7.3 ^{d} ±0.11	6.1 ^{c} ±0.13	7.4 ^{d} ±0.15
Ash	3.5 ^{a} ±0.05	3.9 ^{b} ±0.08	3.6 ^{a} ±0.04	3.6 ^{a} ±0.07	3.5 ^{a} ±0.02	3.5 ^{a} ±0.02

The fatty acid content of the body muscle of juvenile Murray cod, at the commencement of the experiment and of fish reared under different dietary regimes is given in Table 4.19. The fatty acids found in highest concentration amongst the saturates, monoenes and polyunsaturates (PUFAs) were 16:0, 18:1n-9 and 22:6n-3, respectively, and each of these accounted for more than 60% of each of the group's of fatty acids. The muscle fatty acid content was affected by the dietary lipid content. For example the total amount (in μg mg^{-1} lipid) of monoenes ranged from 72±5.1 ($P_{40}L_{10}$) to 112±10.1 ($P_{40}L_{24}$,) and 112±2.8 ($P_{50}L_{17}$) to 132±11.8 ($P_{50}L_{24}$) for the lower and higher protein treatments, respectively, and the n-6 series fatty acids increased with increasing dietary lipid content, though not always significantly between all dietary treatments. Most notably, 18:2n-6 increased with the dietary lipid level in both series of diets. On the other hand, the saturates and n-3 series

fatty acids in body muscle did not appear to show significant changes ($P > 0.05$) with increasing dietary lipid content (Table 4.19).

Table 4.19. The mean (+s.e.) amount of lipid (%) and individual fatty acids in µg mg-1 total lipid in the muscle (fresh) of Murray cod fed different dietary treatments. Each analysis is based on six fish from each replicate. Values with the same superscript in each row are not significantly different (P> 0.05).

Lipid/ F acid	Initial	Dietary treatment				
		P_{40} L_{10}	P_{40} L_{17}	P_{40} L_{24}	P_{50} L_{17}	P_{50} L_{24}
Lipid	1.12^{a} ±0.03	1.23^{a} ±0.04	1.48^{bc} ±0.08	1.22^{a}±0.04	1.34^{ab}±0.01	1.59^{c} ±0.08
14:0	5.6^{ab} ±0.24	4.2^{a} ±0.53	6.9^{b}±0.17	5. 9^{ab}±0.82	6.7^{b}±0.13	7.6^{b}±1.03
16:0	77.1±8.97	81.9±5.27	87.2±0.80	86.4±3.84	93.6±1.65	87.0±4.74
18:0	32.4±4.07	29.3±1.84	30.0±0.40	34.6±0.52	33.2±0.86	30.0±0.97
20:0	0.5^{a} ±0.19	0.6^{a} ±0.12	0.7^{ab}±0.06	1.1^{b}±0.09	0.8^{ab}±0.03	0.9^{ab}±0.07
22:0	0.2^{a} ±0.02	0.2^{a} ±0.01	0.3^{a} ±0.03	0.5^{a} ±0.09	0.6^{a} ±0.11	1.4^{b} ±0.47
Σ sat	116±13.1	116±7.6	125±0.9	128±5.1	135±2.6	127±6.2
16:1n-7	8.9^{a} ±0.66	9.0^{a} ±0.85	15.4^{bc}±0.31	12.2^{ab} ±1.47	14.5^{b} ±0.17	18.1^{c} ±1.50
18:1n-11	3.6^{ab} ±0.41	2.2^{a} ±0.43	4.1^{ab} ±0.58	4.6^{ab} ±0.42	3.8^{ab}±0.40	5.8^{b} ±0.90
18:1n-9	54.6^{ab} ±6.47	43.8^{a} ±2.84	66.8^{b} ±2.04	62.9^{b} ±5.42	65.5^{b} ±2.07	72.3^{b} ±7.31
18:1n-7	9.3±1.07	8.9±0.42	10.7±0.36	10.3±0.63	11.2±0.33	11.7±0.75
20:1n-9	17.5^{c} ±1.26	6.7^{a} ±0.74	12.9^{b} ±0.40	16.2^{bc} ±1.63	12.4^{b} ±0.16	16.1^{bc} ±1.55
Σ mono	97^{b} ±9.8	72^{a} ±5.1	116^{bc} ±2.7	112^{bc} ±10.1	112^{bc} ±2.8	132^{c}±11.8
18:2n-6	18.2^{a} ±1.85	24.1^{a} ±1.82	49.5^{bc}±2.54	50.4^{bc} ±4.94	41.8^{b} ±1.09	55.5^{c} ±5.03
18:3n-3	4.2^{b} ±0.19	2.1^{a} ±0.64	1.7^{a} ±0.40	2.2^{a} ±0.7	2.7^{a} ±0.12	2.2^{a} ±0.36
18:3n-6	25.6±9.84	42.9±3.59	39.4±1.71	35.4±4.01	43.2±2.55	43.7±3.08
18:4n-3	3.5^{a} ±0.06	3.0^{a} ±0.54	4.9^{ab} ±0.14	4.8^{ab} ±0.53	4.8^{ab} ±0.04	6.3^{b} ±0.77
20:2n-6	1.0±0.18	0.9±0.06	1.5±0.22	1.2±0.12	1.0±0.04	1.0±0.15
20:3n-3	1.5±0.11	2.3±0.15	1.4±0.15	2.4±0.67	1.8±0.06	1.7±0.23
20:3n-6	2.3±0.38	2.2±0.66	2.6±0.69	1.6±0.29	1.2±0.14	1.2±0.09
20:4n-6	10.6^{b}±1.52	5.9^{a} ±0.46	5.9^{a} ±0.41	7.0^{a} ±0.17	5.8^{a} ±0.25	5.4^{a} ±0.27
20:5n-3	22.8^{a} ±3.52	25.6^{ab}±2.03	29.7^{bc}±0.24	34.5^{c}±0.99	33.5^{c} ±1.14	31.7^{bc}±1.68
22:5n-3	12.1±1.59	12.8±1.15	14.0±0.48	15.5±0.39	15.4±0.43	14.6±0.77
22:6n-3	76.3±4.81	85.7±5.40	80.2±1.44	89.1±0.71	89.7±0.97	78.7±0.30
Σ n-3	120±10.7	131±9.4	132±2.1	148±3.6	148±2.3	135±4.3
Σ n-6	58^{a} ±13.9	77^{ab}±6.6	99^{b} ±1.8	96^{b}±8.9	93^{b} ±1.1	107^{b}±7.9
Σ PUFA	179^{a} ±24.6	209^{ab} ±16.1	231^{b} ±1.4	244^{b} ±12.2	241^{b} ±1.6	242^{b} ±11.7

4.3.5 Experiment 5: Growth and nutrient utilisation of Murray cod fingerlings fed diets with varying levels of soybean meal and blood meal.

4.3.5.1 Response to the diets

The mean survival and growth data of juvenile Murray cod maintained on the different experimental diets, together with SGR, FCR, PER and digestibility of the diets are given in Table 4.20. In general, the survival of fish on SBM incorporated diets was significantly higher ($P < 0.05$) than for the control diet (CD) or the BM incorporated diets. The level of incorporation of SBM and/or BM did not appear to affect survival with those treatments, however.

Over the 70 day experimental period the mean weight of juvenile Murray cod increased by 313% (16 SBM) to 347% (32 SBM), 221% (32 BM) to 253% (24 BM), and 316%, for the SBM, BM diets, and the CD diets, respectively. The effect of the dietary treatments on body weight became manifested after about the 35th day, when it was evident that Murray cod on the BM diets did not gain weight as rapidly as the control or those on SBM diets (Fig. 4.2). In the case of the 16 BM and 32 BM dietary treatments the fish lost weight after the 56th day. The final mean weight of fish maintained on the CD and all of the SBM dietary treatments were significantly higher ($P < 0.05$) than those on the BM dietary treatments (Table 4.20).

The highest SGR of 1.78 % day^{-1} was recorded for 32 SBM diet, but was not significantly different ($P > 0.05$) from the other SBM diets or the CD. Feed utilisation parameters, such as FCR and PER also reflected similar

trends. For example, the lowest FCR of 1.36 and the highest PER of 1.48 were recorded for the 32 SBM diet. The PCE was also recorded for the 32 SBM diet, and it ranged from 23.1±1.39% (32 SBM) to 17.9±0.22% (16 SBM) for the SBM diets, compared to 9.3±0.82% (32 BM) to 12.23±0.34% (24 BM) for BM diets. However, FCR, PER and PCE recorded for the other SBM diets as well as the CD did not always differ significantly ($P > 0.05$) from each other. On the other hand, significant differences were evident between SBM and BM diets, most notably in the PCE, which was considerably lower than for the SBM diets.

The HSI was lowest (1.08±0.08 %) in Murray cod maintained on the CD, but it did not differ significantly ($P > 0.05$) from that for fish fed the SBM diets. The HSI of fish on SBM diets appeared to decrease with increasing dietary SBM content.

4.3.5.2 Diet Digestibility

During the experiment it was noted that the faecal material ejected by fish on the BM diets tended to be black, and often powdery, indicating that at least a proportion of BM particles were ejected without being digested. This tendency appeared to increase with increasing BM level in the diets.

The ADMD and PD of the CD and the diets containing SBM was high, ranging from 70.6±1.5 to 74.3±1.6 % and from 88.6±0.6 to 91.3±0.6 % respectively, and were not significantly different ($P > 0.05$) for the different SBM diets (Table 4.20). Although, the ADMD did not differ significantly amongst the dietary treatments, a distinct trend was apparent in that of the BM diets which were much lower. The BM diets were relatively poorly digested, when the ADMD ranged from 50.5±4.3 (24 BM) to 69.7±3.5 % (8 BM), and PD from 30.4±20.7 (32 BM) to 74.4±3.0 % (8 BM), respectively. It was also noticeable that the coefficient of variation (CoV- data not shown) for both ADMD and PD estimations of BM diets were considerably higher than for SBM and CD diets, and in general there was a tendency for the CoV of the estimations to increase with increasing level of BM in the diet. For example, the CoV increased from 5.1 to 27.9 %, and from 4.0 to 68.1 % for ADMD and PD estimations for the 8 BM and 32 BM diets, respectively.

4.3.5.3 Carcass composition

The carcass proximate composition of juvenile Murray cod fed different diets is given in Table 4.21. In general, there was a tendency (but not statistically significant) for carcass protein to increase with SBM inclusion level in the diets, but not so with BM diets. Also, carcass protein content was always lower in fish on the BM diets than on the SBM diets, not necessarily significantly in all instances (Table 4.21). The lowest carcass lipid was found in the initial samples (3.56±0.13 %) and the differences amongst the dietary treatments were less marked than in the case of protein. Similarly, the ash content in relation to dietary treatment showed the least difference.

4.3.6 Experiment 6: Evaluation of experimental diets under commercial scale

The feeds used in the growth trials were of similar energy content, but the laboratory formulated diets (DU1 and DU2) had significantly lower lipid content and higher protein content (Table 4.7). The amino acid composition of the diets were similar, except for minor differences in the amount of individual amino acids in the diets, and the significantly lower ($P < 0.05$) amount of NEAA in the CD/B diet (Table 4.8). With regard to the individual amino acids the greatest difference was observed in the significantly lower amount of lysine (280±28.3 in μ moles g feed $^{-1}$ as fed basis) in the CD/B than in the other three diets. The fatty acid composition of the four diets were comparable (Table 4.9), but the DU1 and DU2 had lower amounts of saturates, n- 3, PUFA and HUFA, not always significantly different from the two commercial diets (CD/S and CD/B), however. Some physical differences in the diets were noted during the trials. DU1 tended to float more than CD/S, while DU2 was less water stable (broke up quicker in the water) than did CD/B.

During Trial 1, Murray cod doubled in weight in less than 60 days. Although growth appeared to be slightly greater in fish fed CD/S diet, changes in mean weight (Fig. 4.3) and total length over time were not significantly effected by diet ($P > 0.05$). Diet also did not significantly ($P > 0.05$) effect survival, SGR, FCR, and/ or PER (Table 4.22). However, both mean final weight and condition factor were significantly greater ($P < 0.05$) for fish fed CD/S. Murray cod fed the CD/S diet also had a significantly greater ($P<0.05$) feed rate than did fish fed the DU1 diet (Table 4.22).

Table 4.20. Mean survival rate, final mean weight and different growth related parameters, together with the digestibility estimates of the diets for juvenile Murray cod in response to different experimental diets. The dietary codes are as in Table 4.5. Values in rows with the same superscript are not significantly different (P> 0.05).

Parameter	Dietary Treatment								
	CD	8 SBM	16 SBM	24 SBM	32 SBM	8 BM	16 BM	24 BM	32 BM
Survival (%)	$57.8^{b} \pm 1.3$	$83.7^{c} \pm 1.5$	$93.4^{c} \pm 1.3$	$94.8^{c} \pm 3.2$	$94.1^{c} \pm 0.7$	$29.9^{a} \pm 3.9$	$23.5^{a} \pm 5.3$	$38.0^{a} \pm 4.3$	$30.4^{a} \pm 6.6$
Final Wt. (g)	$10.2^{a} \pm 0.4$	$10.7^{a} \pm 0.3$	$10.0^{a} \pm 0.3$	$10.8^{a} \pm 0.3$	$11.4^{a} \pm 0.3$	$7.8^{b} \pm 0.4$	$7.7^{b} \pm 0.5$	$8.3^{b} \pm 0.4$	$7.2^{b} \pm 0.3$
Wt. Increase (%)	$316.8^{bcd} \pm 20.8$	$339.0^{d} \pm 26.5$	$313.8^{bcd} \pm 13.9$	$326.3^{cd} \pm 14.1$	$347.8^{d} \pm 25.5$	$241.7^{abc} \pm 9.8$	$233.6^{ab} \pm 18.8$	$253.0^{abc} \pm 4.7$	$221.8^{a} \pm 9.2$
SGR (% day^{-1})	$1.65^{bcd} \pm 0.09$	$1.74^{d} \pm 0.11$	$1.63^{bcd} \pm 0.06$	$1.69^{cd} \pm 0.06$	$1.78^{d} \pm 0.10$	$1.26^{ab} \pm 0.06$	$1.21^{a} \pm 0.12$	$1.33^{abc} \pm 0.03$	$1.14^{a} \pm 0.06$
FCR	$1.54^{a} \pm 0.02$	$1.36^{a} \pm 0.08$	$1.45^{a} \pm 0.07$	$1.42^{a} \pm 0.11$	$1.36^{a} \pm 0.08$	$2.10^{b} \pm 0.16$	$2.06^{b} \pm 0.13$	$2.12^{b} \pm 0.06$	$2.51^{b} \pm 0.19$
PER	$1.30^{bc} \pm 0.02$	$1.48^{c} \pm 0.08$	$1.38^{c} \pm 0.07$	$1.42^{c} \pm 0.12$	$1.48^{c} \pm 0.09$	$0.96^{ab} \pm 0.07$	$0.98^{ab} \pm 0.06$	$0.95^{ab} \pm 0.03$	$0.81^{a} \pm 0.07$
PCE	$17.90^{b} \pm 0.22$	$20.56^{bc} \pm 1.13$	$17.90^{b} \pm 0.22$	$21.56^{bc} \pm 1.71$	$23.07^{c} \pm 1.39$	$12.16^{a} \pm 0.94$	$11.14^{a} \pm 0.82$	$12.23^{a} \pm 0.34$	$9.26^{a} \pm 0.82$
HSI (%)	$2.08^{a} \pm 0.08$	$3.16^{ab} \pm 0.41$	$2.27^{ab} \pm 0.24$	$2.70^{ab} \pm 0.19$	$2.51^{ab} \pm 0.10$	$2.50^{ab} \pm 0.10$	$3.49^{bc} \pm 0.41$	$5.25^{c} \pm 0.63$	$3.53^{bc} \pm 0.37$
Digestibility (%)									
ADMD	74.3 ± 1.63	70.9 ± 1.45	70.6 ± 1.46	71.3 ± 0.50	72.3 ± 1.81	69.7 ± 3.53	52.4 ± 7.63	50.5 ± 4.27	51.6 ± 14.4
PD	$91.3^{c} \pm 0.55$	$88.6^{c} \pm 0.57$	$89.8^{c} \pm 0.50$	$90.3^{c} \pm 0.17$	$90.8^{c} \pm 0.60$	$74.4^{bc} \pm 2.99$	$50.3^{ab} \pm 7.96$	$31.0^{a} \pm 5.95$	$30.4^{a} \pm 20.7$

Table 4.21. Carcass proximate composition in percent wet weight (mean values ± s.e). The dietary codes are as in Table 4.5. Values in rows with same superscript are not significantly different (P> 0.05).

Parameter	Initial	Dietary treatment								
		CD	8 SBM	16 SBM	24 SBM	32 SBM	8 BM	16 BM	24 BM	32 BM
Moist.	$78.8^{bc} \pm 0.13$	$77.0^{a} \pm 0.55$	$76.2^{abc} \pm 0.22$	$76.7^{a} \pm 0.22$	$76.4^{a} \pm 0.24$	$77.1^{abc} \pm 0.17$	$77.7^{abc} \pm 1.08$	$78.4^{abc} \pm 0.45$	$77.3^{abc} \pm 0.46$	$79.2^{c} \pm 0.56$
Protein	$14.0^{dc} \pm 0.09$	$13.9^{bcd} \pm 0.09$	$13.9^{bcd} \pm 0.18$	$14.9^{de} \pm 0.31$	$14.7^{de} \pm 0.39$	$15.1^{e} \pm 0.34$	$13.3^{abc} \pm 0.12$	$12.6^{a} \pm 0.04$	$13.4^{abc} \pm 0.16$	$12.8^{ab} \pm 0.10$
TL	$3.6^{a} \pm 0.13$	$5.1^{bc} \pm 0.16$	$4.8^{bc} \pm 0.17$	$5.4^{c} \pm 0.09$	$5.5^{c} \pm 0.06$	$4.3^{ab} \pm 0.22$	$4.4^{ab} \pm 0.10$	$4.7^{bc} \pm 0.19$	$5.0^{bc} \pm 0.25$	$4.3^{ab} \pm 0.36$
Ash	$3.5^{abc} \pm 0.06$	$3.6^{abc} \pm 0.06$	$3.5^{abc} \pm 0.15$	$3.3^{ab} \pm 0.04$	$3.5^{abc} \pm 0.12$	$3.4^{a} \pm 0.02$	$3.7^{c} \pm 0.02$	$3.6^{abc} \pm 0.05$	$3.7^{c} \pm 0.02$	$3.6^{bc} \pm 0.02$
Energy kJ/g*	35.4	31.7	32.3	38.9	32.8	31.2	32.2	32.6	33.0	32.1

* Mean of duplicate samples

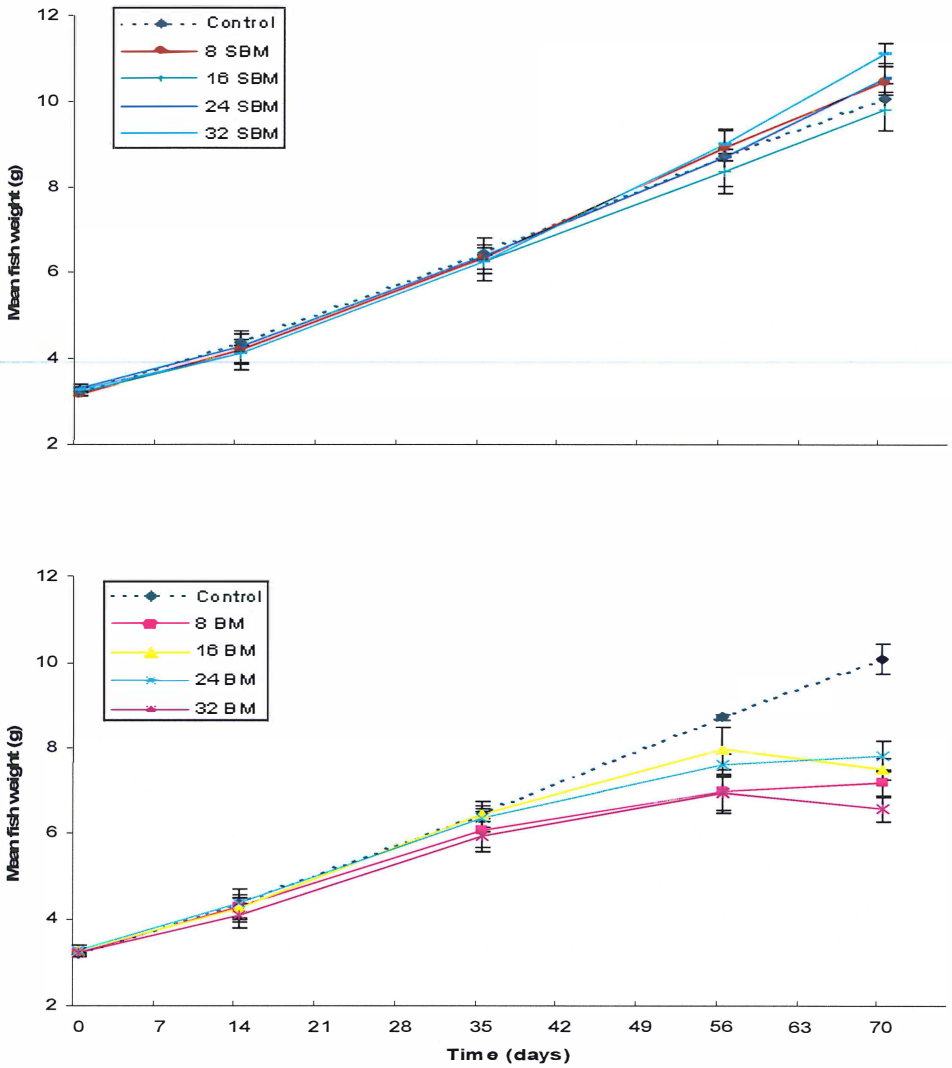

Fig. 4.2. The mean (± s.e.) weight of juvenile Murray cod reared under different dietary treatments. Upper: increase in mean weight of Murray cod juveniles fed the control diet and the soybean meal (SBM) substituted diets. Lower: increase in mean weight of Murray cod juveniles fed the control diet and the blood meal (BM) substituted diets

Murray cod in Trial 2 doubled their weight in 66 days and 78 days for fish fed the DU2 and the CD/B diets, respectively. Growth was greatest in fish fed the DU2 (Fig. 4.3), but change in mean weight over time was not significantly effected by diet ($P > 0.05$), whereas changes in total length over time were significantly greater for fish fed the DU2 diet ($P < 0.05$). As in Trial 1 diet did not significantly ($P > 0.05$) effect survival, SGR, FCR, PER, condition factor or feed rate (Table 4.22). However, both mean final weight and final total length were significantly greater ($P < 0.01$) for fish fed DU2. Condition factor at the end of Trial 2 was significantly greater than at the beginning for fish fed both diets.

The highest HSI (2.1±0.11) was observed in fish fed the CD/B diet, and was significantly higher than in other treatments except that of initial fish in Trial 1 (Table 4.22). In both feed trials the lowest HSI was observed in fish fed diets DU1 (1.3±0.05) and DU2 (1.4±0.05).

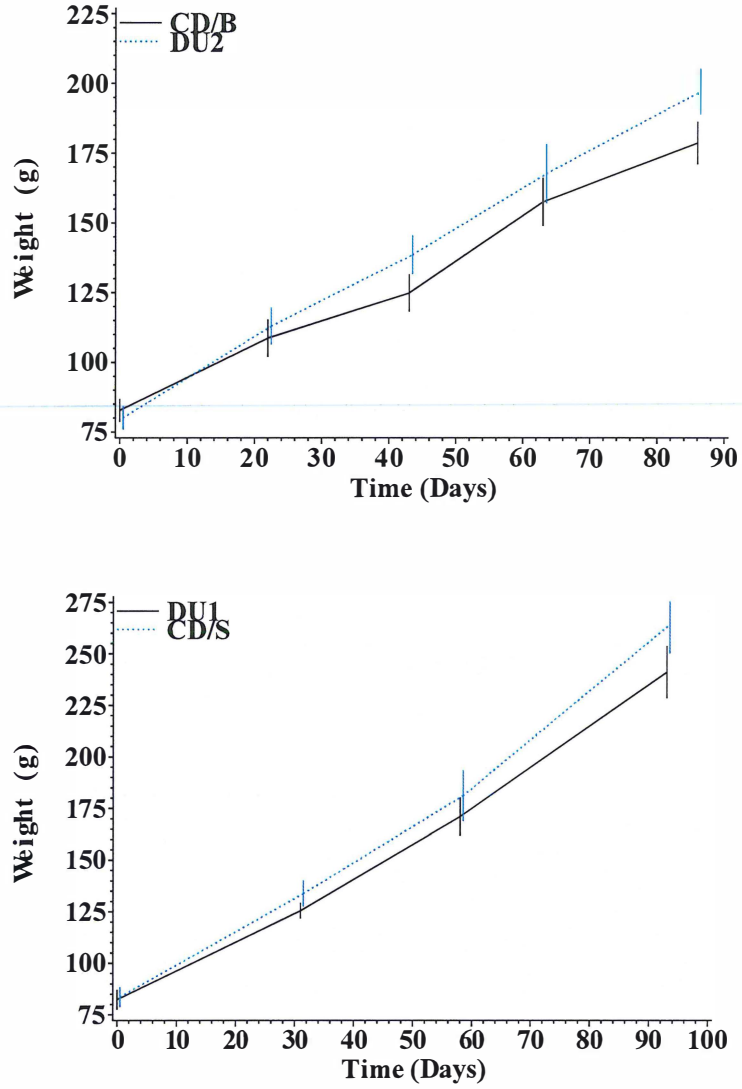

Fig. 4.3. Growth in weight (mean + s.e.) of Murray cod fed different diets during Experiment 6. Upper: Trial 1, and Lower: Trial 2.

Table 4.22. Growth and feed utilisation indices (± s.e.) for Murray cod reared on different diets in the two trials. The initial mean weight and total length at each trial are also given. Values in the same row with the different superscripts (ab-Trial 1 and xy-Trial 2) are significantly different (P< 0.05).

Parameter	Trial 1 (2000)		Trial 2 (2001)	
	DU1	CD/S	DU2	CD/B
Weight (g)				
Initial	82.3 ± 2.3	83.5 ± 2.3	80.0 ± 2.0	82.8 ± 2.0
Final	241.1 ± 6.2^{a}	264.0 ± 7.0^{b}	197.0 ± 4.1^{y}	178.7 ± 3.8^{x}
Total length (mm)				
Initial	182 ± 1.5	183 ± 1.6	183 ± 1.6	185 ± 1.4
Final	258 ± 1.9	263 ± 1.9	243 ± 1.4^{y}	235 ± 1.4^{x}
Condition factor				
Initial	1.36 ± 0.02	1.36 ± 0.02	1.29 ± 0.01	1.29 ± 0.01
Final	1.38 ± 0.02^{a}	1.43 ± 0.01^{b}	1.35 ± 0.01	1.36 ± 0.01
Survival (%)	98.4 ± 0.4	99.6 ± 0.2	99.7 ± 0.1	99.5 ± 0.2
Feed rate (% day^{-1})	1.09 ± 0.02^{a}	1.24 ± 0.01^{b}	0.86 ± 0.03	0.94 ± 0.02
SGR (% day^{-1})	1.16 ± 0.01	1.24 ± 0.06	1.05 ± 0.04	0.89 ± 0.05
FCR	0.92 ± 0.03	1.00 ± 0.01	0.85 ± 0.04	1.09 ± 0.09
PER (%)	2.23 ± 0.08	2.16 ± 0.02	2.40 ± 0.13	2.08 ± 0.16
HSI	1.3 ± 0.05^{a}	1.7 ± 0.25^{b}	1.4 ± 0.05^{x}	2.1 ± 0.11^{y}

The approximate commercial purchase price of CD/S, CD/B and DU2 are $AUD 1,800 tonne^{-1}, $AUD 1,670 tonne^{-1} and $AUD $1,540 tonne^{-1}, respectively (indicative quoted prices May 2002). Based on these prices, combined with FCR values for each diet (Table 4.22), the cost of feed per tonne of fish produced was lowest for the DU2 diet ($AUD 1,310 tonne^{-1}), which was $AUD 490-500 tonne^{-1} cheaper than either of the other diets (CD/S $AUD 1,810 tonne^{-1}; CD/B $AUD 1,820 tonne^{-1}).

The proximate composition of muscle and carcass of Murray cod fed different diets in the current study is given in Table 4.23, together with statistical results for comparison of each of the parameters within each trial and between trials. In both feed trials, for all diets, lipid levels in muscle were significantly lower (P< 0.05) than fish at the commencement of the experiment, but carcass lipid content was higher. Also, the muscle and carcass lipid content (in percent wet weight) in Murray cod maintained on diets DU1 (1.08±0.07 and 7.3±0.14) and DU2 (0.99±0.05 and 7.9±0.20) were lower, and significantly (P< 0.05) so in the latter, than in fish on the corresponding diets, CD/ S (1.10±0.02 and 8.9±0.02) and CD/B (1.02±0.04 and 8.8±0.07), respectively (Table 4.23).

With regard to the fatty acid composition of muscle in Murray cod fed different diets, the lowest amounts (in µg mg lipid^{-1}) of n- 3 (262.5± 2.9), n- 6 (39.8± 0.9) and PUFA (302.3± 3.8) were observed (P< 0.05) in fish reared on the salmon diet (CD/S), and the highest in fish reared on DU2 and CD/B diets (Table 4.24). The differences in the amount of HUFA and the n- 3 to n- 6 ratio in muscle of fish reared on different diets were not significant (P> 0.05), except in fish on the two commercial diets.

Even though there were minor differences in the amount of individual fatty acids in Murray cod on different dietary treatments and in initial fish, the fatty acids that were dominant in each class were similar amongst the different groups. For example, 16:0 and 18:0, 18:1n- 9 and 16:1n- 7, and 22:6n- 3, 20:5n- 3, 22:5n- 3 and 18:2n- 6 were the dominant fatty acids amongst the saturates, monoenes and PUFA, respectively in all samples (Table 4.24). These eight fatty acids accounted for between 80.8 and 88.7 % of all identified fatty acids (23) in muscle of Murray cod on different dietary treatments.

The atherogenicity (AI) and thrombogenicity (TI) indices of Murray cod reared under different dietary treatments were not widely different in the different groups. The AI ranged from 1.24 (DU2) to 1.43 (CD/S), and TI from 0.23 (DU2) to 0.26 (CD/S).

Table 4.23. The mean (+ s.e.) percent moisture, protein, lipid and ash in the muscle and carcass, and HSI values of Murray cod fed different diets (data are given on a fresh basis). Values in the same row with the same superscripts (abc-all data) or subscripts (klm-trial 1; xyz-trial 2) are not significantly different (P> 0.05).

Parameter	Trial 1 (2000)			Trial 2 (2001)		
(%)	Initial	DU1	CD/S	Initial	DU2	CD/B
Muscle						
Moisture	77.4 ± 0.01^{ak}	79.2 ± 0.20^{bl}	77.9 ± 0.34^{ak}	79.2 ± 0.55^{b}	78.4 ± 0.15^{ab}	78.4 ± 0.21^{ab}
Protein	18.8 ± 0.03^{bckl}	18.6 ± 0.06^{bk}	19.1 ± 0.03^{cl}	18.0 ± 0.09^{ax}	18.8 ± 0.13^{bcy}	18.5 ± 0.02^{by}
Lipid	1.26 ± 0.07^{bl}	1.08 ± 0.07^{ak}	1.0 ± 0.02^{ak}	1.32 ± 0.01^{by}	0.99 ± 0.05^{ax}	1.02 ± 0.04^{ax}
Ash	1.16 ± 0.00^{bk}	1.19 ± 0.01^{cl}	1.25 ± 0.01^{dm}	1.12 ± 0.0^{by}	1.15 ± 0.01^{by}	1.08 ± 0.0^{ax}
Carcass						
Moisture	70.6 ± 0.59^{ab}	71.1 ± 1.18^{ab}	67.1 ± 0.95^{a}	72.2 ± 0.23^{b}	69.7 ± 0.72^{ab}	69.3 ± 0.95^{ab}
Protein	15.7 ± 0.21^{ak}	15.2 ± 0.42^{ak}	16.6 ± 0.06^{bl}	16.4 ± 0.04^{b}	17.2 ± 0.28^{b}	16.5 ± 0.17^{b}
Lipid	5.5 ± 0.02^{ak}	7.3 ± 0.14^{bl}	8.9 ± 0.02^{dm}	7.2 ± 0.13^{bx}	7.9 ± 0.20^{cy}	8.8 ± 0.07^{dz}
Ash	3.7 ± 0.12^{ab}	3.7 ± 0.15^{ab}	4.1 ± 0.04^{b}	3.6 ± 0.07^{ab}	3.4 ± 0.17^{ab}	3.2 ± 0.18^{a}
HSI	1.9 ± 0.15^{bcl}	1.3 ± 0.05^{ak}	1.7 ± 0.25^{abckl}	1.6 ± 0.09^{abcx}	1.4 ± 0.05^{abx}	2.1 ± 0.11^{cy}

Table 4.24. The mean (± s.e.) amount of fatty acids in muscle (µg mg lipid^{-1}) of Murray cod fed different diets (data are given on a fresh basis). Values in the same row with the same superscripts (abc-all data) or subscripts (klm-trial 1; xyz-trial 2) are not significantly different (P> 0.05).

Fatty acids	Trial 1 (2000)			Trial 2 (2001)		
	Initial	DU2	CD/S	Initial	DU2	CD/B
14:0	22.5±1.2$^{b}_{l}$	14.8±0.7$^{a}_{k}$	15.0±1.1$^{a}_{k}$	18.8±0.1$^{ab}_{xy}$	23.3±2.1$^{b}_{y}$	15.5±0.2$^{a}_{x}$
16:0	188.5±3.0^{c}	184.1±2.9^{c}	175.4±4.9^{bc}	147.7±0.1$^{a}_{x}$	180.6±3.6$^{bc}_{y}$	169.6±2.1$^{b}_{y}$
18:0	58.3±2.2$^{c}_{l}$	50.1±1.7$^{ab}_{k}$	55.3±0.6$^{bc}_{kl}$	45.3±0.3$^{a}_{x}$	48.8±2.0$^{ab}_{xy}$	53.8±1.4$^{bc}_{y}$
20:0	1.3±0.0	1.5±0.0	1.3±0.0	1.4±0.0	1.9±0.1	2.1±0.2
22:0	0.5±0.0	0.6±0.1	0.5±0.0	0.5±0.0	0.5±0.1	0.4±0.1
Σ saturates	271.4±4.5$^{c}_{l}$	251.2±3.9$^{b}_{k}$	247.7±6.8$^{b}_{k}$	213.3±0.5$^{a}_{x}$	255.2±4.9$^{b}_{y}$	241.2±3.4$^{b}_{y}$
16:1n- 7	46.3±1.8$^{bc}_{l}$	32.7±0.9$^{a}_{k}$	32.2±1.8$^{a}_{k}$	44.5±0.0$^{abc}_{xy}$	53.4±4.6$^{c}_{y}$	37.8±1.1$^{ab}_{x}$
18:1n- 9	115.2±3.1$^{b}_{l}$	100.0±0.3$^{a}_{k}$	87.1±3.0$^{a}_{k}$	88.3±0.3^{a}	99.9±5.4^{a}	89.8±0.4^{a}
18:1n- 7	21.4±0.3$^{b}_{l}$	16.0±0.1$^{a}_{k}$	15.9±0.5$^{a}_{k}$	19.7±0.1^{b}	21.4±0.7^{b}	19.8±0.2^{b}
20:1n- 9	9.0±0.4$^{c}_{l}$	18.7±0.2$^{d}_{m}$	7.7±0.3$^{b}_{k}$	5.0±0.0^{a}	5.4±0.5^{a}	4.2±0.1^{a}
22:1n- 11	1.3±0.2$^{a}_{k}$	14.4±0.6$^{c}_{m}$	3.2±0.0$^{b}_{l}$	0.6±0.0^{a}	0.9±0.1^{a}	0.5±0.0^{a}
Σ monoenes	193.3±5.9$^{c}_{l}$	181.9±1.7$^{bc}_{l}$	146.3±5.8$^{a}_{k}$	158.3±0.4^{ab}	181.3±11.1^{bc}	152.3±1.2^{ab}
18:2n- 6	24.2±0.8$^{bc}_{l}$	20.4±0.2$^{ab}_{kl}$	17.0±0.6$^{a}_{k}$	21.3±0.1$^{ab}_{x}$	27.1±1.7$^{c}_{y}$	19.6±0.2$^{ab}_{x}$
18:3n- 3	8.8±0.3$^{c}_{l}$	7.7±0.2$^{bc}_{kl}$	6.9±0.6$^{ab}_{k}$	6.0±0.3$^{a}_{x}$	7.4±0.4$^{bc}_{y}$	5.7±0.1$^{a}_{x}$
18:3n- 6	4.1±0.2$^{b}_{l}$	2.1±0.0$^{a}_{k}$	2.9±0.4$^{ab}_{kl}$	2.6±0.1^{a}	3.4±0.4^{ab}	2.4±0.1^{a}
18:4n- 3	14.6±0.4^{bc}	10.3±0.5^{ab}	9.6±0.4^{ab}	11.5±0.6$^{ab}_{x}$	16.7±1.8$^{c}_{y}$	8.9±0.3$^{a}_{x}$
20:2n- 6	2.0±0.0	2.1±0.0	1.6±0.0	1.6±0.1	2.3±0.2	1.7±0.1
20:3n- 3	1.0±0.0	1.9±0.0	1.9±0.4	1.4±0.2	1.3±0.1	1.8±0.1
20:3n- 6	1.4±0.0	1.0±0.0	1.1±0.0	1.2±0.0	1.5±0.2	1.1±0.0
20:4n- 6	15.6±0.6	13.4±0.7	15.1±0.0	13.2±0.1	12.9±1.0	14.2±0.5
20:5n- 3	74.1±0.7$^{cd}_{m}$	56.9±0.8$^{a}_{k}$	65.9±1.8$^{b}_{l}$	72.6±0.1$^{cd}_{xy}$	77.3±1.3$^{d}_{y}$	70.0±1.2$^{bc}_{x}$
22:4n- 6	2.0±0.1^{ab}	1.8±0.1^{a}	1.7±0.1^{a}	2.4±0.1^{abc}	2.7±0.2^{bc}	3.0±0.1^{c}
22:3n- 3	4.6±0.2$^{a}_{k}$	4.5±0.0$^{a}_{k}$	4.9±0.1$^{b}_{l}$	<0.1	<0.1	<0.1
22:5n- 3	28.1±0.3$^{c}_{l}$	21.8±0.2$^{a}_{k}$	23.4±0.6$^{ab}_{k}$	25.1±0.0$^{b}_{x}$	27.9±0.9$^{c}_{y}$	25.1±0.3$^{b}_{x}$
22:6n- 3	158.6±5.3	166.5±7.4	149.6±0.8	155.2±0.5	169.0±7.8	173.3±5.6
Σ n- 3	290.1±5.7$^{bc}_{l}$	269.9±7.7$^{ab}_{kl}$	262.5±2.9$^{a}_{k}$	272.0±1.0$^{ab}_{x}$	299.8±5.0$^{c}_{y}$	285.0±7.0$^{abc}_{xy}$
Σ n- 6	49.6±0.4$^{b}_{l}$	41.4±0.6$^{a}_{k}$	39.8±0.9$^{a}_{k}$	42.5±0.1$^{a}_{x}$	50.1±1.8$^{b}_{y}$	42.2±0.6$^{a}_{x}$
Σ PUFA	339.7±5.5$^{bc}_{l}$	311.3±8.4$^{a}_{k}$	302.3±3.8$^{a}_{k}$	314.5±1.1$^{ab}_{x}$	350.0±5.6$^{c}_{y}$	327.2±7.6$^{abc}_{xy}$
Σ HUFA	287.9±7.1	270.6±9.5	265.8±2.9	272.9±0.7	295.2±7.7	290.5±7.8
n- 3 : n- 6	5.8±0.1ak	6.5±0.1abkl	6.6±0.1bl	6.3±0.0ab	6.0±0.2ab	6.7±0.0b

4.3.7 Experiment 7: Comparisons of the fillet quality of cultured and wild Murray cod

The proximate composition and fatty acid composition of muscle of wild and farmed Murray cod, harvested in 2001, are given in Table 4.25 and Table 4.26, respectively. The biggest difference in the proximate composition was observed in the lipid content, which was significantly higher ($P< 0.05$) in farmed fish. The protein content of muscle was significantly higher only in purged fish compared to the others. Indeed, in purged fish, protein, lipid and ash content was significantly higher ($P< 0.05$) than in wild fish as well as in pre- purged fish from the same harvest.

Major differences in the fatty acid profiles of wild and farmed Murray cod were evident (Table 4.26). Wild Murray cod had significantly less ($P< 0.05$) saturates (192.6±1.84 vs 266.3±3.51), monoenes (156.5±8.7 vs 207.6±6.19), n- 3 (145.2±5.24 vs 261.8±3.2) but higher n- 6 (144.3±2.73 vs 48.3±1.38) in muscle (all values are in μg mg lipid^{-1}) than in farmed fish. Also, wild fish had a much lower n- 3 to n- 6 ratio (1.0±0.03 vs 5.4±0.09). The above differences were also reflected in differences in individual fatty acids; wild fish having significantly lower amounts of 14:0, 16:0 and 20:0 (amongst saturates), 16:1n- 7, 18:1n- 9, 20:1n- 9 and 22:1n- 11 (amongst monoenes), 18:4n- 3, 20:5n –3 and 22:6n- 3 (amongst PUFA), but had significantly higher amounts of 18:1n- 7, 18:2n- 6, 20:3n- 3, 20:3n- 6 and 22:4n– 6. Generally, the amount of most fatty acids was slightly lower in purged fish than in non-purged fish but not significantly so.

The atherogenicity (AI) and thrombogenicity (TI) indices of wild and farmed Murray cod ranged from reared 0.96 (wild fish) to 1.32 (farmed fish) and 0.27 (farmed fish) to 0.29 (wild fish), respectively.

Table 4.25. The mean (+ s.e.) percent moisture, protein, lipid and ash in the muscle (fresh basis) of wild and farmed Murray cod. Values in the same row with the same superscripts are not significantly different (P> 0.05). Comparisons are made on the basis of the mean values for wild fish. PreP- pre purge; PostP- post purged.

Parameter	Wild-1	Wild -2	Wild mean	Farmed		
				2000 PreP	2001 PreP	2001 PostP
Moisture	77.4±0.4	79.8±1.1	78.6±0.4^{b}	77.1±0.4^{ab}	75.3±0.7^{a}	75.5±0.4^{a}
Protein	19.7±0.1	18.6±0.0	19.2±0.2^{a}	19.9±0.1^{ab}	19.2±0.3^{a}	20.4±0.1^{b}
Lipid	0.72±0.04	0.69±0.02	0.7±0.02^{a}	1.26±0.06^{b}	1.70±0.05^{c}	2.18±0.25^{d}
Ash	1.04±0.01	1.01±0.01	1.03±0.01^{a}	1.22±0.03^{b}	1.18±0.05^{b}	1.36±0.04^{c}

4.4 Discussion

4.4.1 Experiment 1. Determination of the optimal dietary protein requirement in juvenile Murray cod

Protein requirements vary between species, with carnivorous fish generally having a higher dietary protein requirement than omnivorous and herbivorous species (Wilson 1991; National Research Council 1993). Knowledge about the protein requirements is important for diet development, because feed costs usually exceed 50% of production costs in intensive aquaculture practices, and protein is the costliest component of feeds.

Murray cod is a top order carnivore (Harris and Rowland 1996), and its protein requirement estimated from the present study is comparable to that reported for other carnivorous species (Wilson 1991; National Research Council 1993). The present study was based on a dose-response design, and growth increased with increasing dietary protein content up to a point, and growth decreased thereafter with further increases in dietary protein content. The best growth response together with the best FCR was observed in fish on diet P50. The digestible energy (DE) values of the diets were 13.8 (P40), 13.3 (P45), 16.3 (P50), 16.4 (P55) and 15.7 (P60) kJ g^{-1}, and the DE g protein^{-1} (kJ g protein^{-1}) for the diets were 33.8, 29.5, 33.3, 30.1, 26.6, in order. The results indicate that for Murray cod juveniles, diets exceeding 16.3 kJ g^{-1} do not necessarily have a significant influence on growth. Also, rate of growth did not show an apparent trend in relation to DE g protein^{-1}.

Table 4.26. The mean (+ s.e.) amount of fatty acids in the muscle (µg mg lipid-1) of wild and farmed Murray cod. Values in the same row with the same superscripts are not significantly different (P> 0.05). Comparisons are made on the basis of the mean values for wild fish. PreP- pre purged; PostP- Post purged.

Fatty acids	Wild-1	Wild -2	Wild mean	Farmed		
				2000 PreP	2001 PreP	2001 PostP
14:0	6.1±0.91	3.2±0.2	4.4±0.44^{a}	22.7±1.17^{b}	29.1±2.0^{c}	28.3±1.6^{c}
16:0	133.2±1.78	132.2±1.48	132.6±1.10^{a}	176.4±5.32^{b}	185.7±2.07^{b}	184.9±3.61^{b}
18:0	48.0±0.81	57.9±0.47	53.6±1.42	50.0±2.08	48.4±0.48	49.6±0.50
20:0	0.9±0.05	1.3±0.02	1.2±0.06^{a}	1.8±0.10^{b}	2.1±0.04^{c}	2.2±0.03^{c}
22:0	0.4±0.05	0.7±0.06	0.63±0.05	0.7±0.06	0.8±0.08	0.6±0.08
Σ sat	188.7±2.89	195.5±1.93	192.6±1.84^{a}	251.8±7.62^{b}	266.3±3.51^{b}	265.8±4.83^{b}
16:1n- 7	45.3±1.64	24.5±1.39	33.4±3.02^{a}	43.8±1.59^{b}	58.5±2.19^{c}	55.2±3.06^{c}
18:1n- 9	116.0±6.99	82.2±1.07	96.7±5.47^{a}	108.1±3.81^{ab}	118.4±3.3^{b}	116.7±4.61^{b}
18:1n- 7	23.6±0.79	23.3±0.85	23.4±0.57^{c}	17.7±0.64^{a}	21.4±0.43^{b}	21.0±0.78^{b}
20:1n- 9	3.1±0.34	1.7±0.04	2.3±0.23^{a}	8.0±0.99^{b}	7.9±0.36^{b}	7.8±0.55^{b}
22:1n- 11	0.5±0.05	0.5±0.05	0.5±0.03^{a}	0.8±0.10^{b}	1.1±0.07^{b}	1.0±0.1^{b}
Σ mono	188.6±9.36	132.4±3.34	156.5±8.7^{a}	178.6±6.76^{ab}	207.6±6.19^{b}	202.0±9.05^{b}
18:2n- 6	30.8±4.40	39.7±1.57	35.9±2.33^{b}	22.6±1.37^{a}	25.7±1.27^{a}	25.6±1.35^{a}
18:3n- 3	15.4±1.32	5.7±0.32	9.9±1.45	7.3±0.47	7.1±0.47	6.7±0.41
18:3n- 6	3.5±0.12	3.9±0.09	3.7±0.08^{b}	2.8±0.11^{a}	3.2±0.11^{a}	3.1±0.11^{a}
18:4n- 3	2.0±0.10	1.1±0.16	1.5±0.15^{a}	12.6±0.79^{b}	15.1±1.04^{b}	14.9±1.06^{b}
20:2n- 6	3.1±0.41	4.3±0.10	3.8±0.24^{b}	1.8±0.14^{a}	1.9±0.12^{a}	1.6±0.07^{a}
20:3n- 3	2.6±0.12	2.3±0.18	2.4±0.11^{b}	1.0±0.1^{a}	0.9±0.1^{a}	0.7±0.07^{a}
20:3n- 6	3.2±0.45	2.8±0.10	2.9±0.20^{b}	1.6±0.13^{a}	1.6±0.08^{a}	1.4±0.07^{a}
20:4n- 6	87.0±2.18	82.1±0.94	84.2±1.23^{c}	17.6±0.88^{b}	13.4±0.48^{a}	14.1±0.48^{a}
20:5n- 3	18.2±1.08	35.8±1.7	28.2±2.65^{a}	79.6±2.53^{b}	84.9±1.61^{b}	85.5±1.21^{b}
22:4n- 6	13.3±0.22	13.9±0.21	13.6±0.17^{b}	2.2±0.11^{a}	2.3±0.13^{a}	2.3±0.09^{a}
22:5n- 3	16.4±0.24	29.2±1.23	23.8±1.89^{a}	26.1±0.78^{a}	32.1±0.93^{b}	31.5±1.0^{b}
22:6n- 3	69.5±1.02	86.6±1.71	79.2±2.56^{a}	123.4±4.06^{b}	121.5±1.78^{b}	113.3±1.36^{b}
Σ n- 3	124.2±0.68	161.0±2.5	145.2±5.24^{a}	250.1±6.8^{b}	261.8±3.2^{b}	252.9±2.4^{b}
Σ n- 6	141.0±5.46	146.8±2.5	144.3±2.73^{b}	48.7±1.77^{a}	48.3±1.38^{a}	48.4±1.01^{a}
Σ PUFA	265.3±5.43	307.8±2.2	289.6±6.35	298.9±8.23	310.2±4.57	301.3±3.45
Σ HUFA	213.4±3.14	257.3±2.7	238.5±6.33	253.5±7.62	258.9±2.51	250.8±0.92
n- 3/ n- 6	0.9±0.03	1.1±0.03	1.0±0.03^{a}	5.2±0.13^{b}	5.4±0.09^{b}	5.2±0.06^{b}

Murai (1992) pointed out the discrepancies that occur in protein requirement studies in fish, and indicated that the primary reason for such discrepancies arise when the EAA requirements are not met. The EAA requirements of Murray cod are not known but the amino acid composition of eggs and larvae are known (Gunasekera *et al.* 1999a). Wilson and Cowey (1985) did not find any differences in the body amino acid composition of rainbow trout (*Oncorhynchus mykiss* Walbaum) and Atlantic salmon (*Salmo salar* L.). Mambrini and Kaushik (1995) reported that the whole body amino acid composition is rather consistent amongst teleosts, and that by and large reflects the EAA requirements of the respective species. The present experimental diets contained all the EAA in excess (data not shown) of that found in Murray cod eggs and larvae (Gunasekera *et al.* 1999a), and/or the known requirements of other carnivorous fish (Wilson 1991; National Research Council 1993). The fact that the best SGR, FCR and PER occurred at a dietary protein level of 50% indicates that 50% dietary protein is optimal for juvenile Murray cod of the levels tested in the present study (at a lipid level of 10%).

It was evident that the protein content in body muscle of Murray cod, by dry weight, was over 90%, and is much higher than that of other cultured species, irrespective of the quality of the diets (Webster *et al.* 1992; Heras *et al.* 1994; Keembiyahetty and Wilson 1998, amongst others). The main reason for this difference was that Murray cod accumulated small amounts of lipid in contrast to other species. In Murray cod the PCE observed were comparable to, if not better than, that reported for most carnivorous species (Kim *et al.* 1991; Keembiyahetty and Wilson 1998). In Murray cod the PCE declined, almost linearly, in relation to increasing dietary protein content. Such a clear-cut trend has not been reported previously (Kim *et al.* 1991), but the

trend has been observed in some species (Millikin 1982).

4.4.2 Experiment 2. Comparison of digestibility and amino acid availability of three protein rich dietary ingredients

Shark meat meal (SMM) is a waste resulting from the extraction of cartilage for the pharmaceutical industry. In Victoria, Australia, at the time this research was conducted, 7-12 metric tonne per week of shark meat waste was produced, costing the processors $AUD 50-65 per mt for disposal (Peter Wright, Zootech, Victoria, Australia. *Pers comm*). Åsgård and Austreng (1985) reported high digestibility of dogfish (*Squalus acanthias*) offal by salmonids and demonstrated its suitability for use in salmonid diets. Heras *et al.* (1994) also reported positively on the use of dogfish silage in diets for Atlantic salmon. The current study showed that SMM was almost completely digested by Murray cod. As such SMM waste has the potential to be used in practical diets for Murray cod, and further experiments are needed to determine the optimal amount that can be incorporated in to practical diets for these two species.

SBM was relatively well digested by Murray cod, as in the case of most finfish (Webster *et al.* 1992; Sadiku and Jauncey 1995; Boonyaratapalin *et al.* 1998). Apart from certain anti-nutritional properties (protease inhibitors) of SBM, Baeverfjord and Krogdahl (1996) reported that SBM induced enteritis in Atlantic salmon, and the condition reversed rather quickly, when transferred on to a diet lacking SBM. All this suggest that SBM may have to be used with a certain degree of caution, and further research is warranted on the optimal levels of dietary inclusion in cultured finfish species.

Generally, high levels of ash and/or fibre in feed stuffs (Cho and Slinger 1979; De Silva 1985; McGoogan and Reigh 1996), and the nitrogen-free extract (NFE) fraction (Gaylord and Gatlin 1996) result in low ADM and PD. Similarly, the PD of an ingredient is not necessarily a reflection of the protein content of the ingredient (Nengas *et al.* 1995), although most ingredients with a high protein content results in high PD (McGoogan and Reigh 1996). The reduced PD of ingredients with high ash and/or fibre content has been attributed to a reduction of the activity of proteases (Falge *et al.* 1978) and shortened evacuation time (Steffens 1989). The lower dry matter, protein and energy digestibility of MM by Murray cod may have been due to the relatively high ash content. SBM on the other hand had lower ash content but the highest NFE of the ingredients tested. It is generally accepted that carnivorous fish have only a limited ability to digest feeds of high carbohydrate content, resulting in a lower energy digestibility of SBM in most instances, as was the case with Murray cod. It is therefore evident that different factors, singly or collectively, may influence the digestibility of an ingredient. In this regard, Gaylord and Gatlin (1996) pointed out that comparisons of organic matter digestibility values of feed stuffs of animal origin with those of plant origin might be problematic.

It needs to be pointed out that in the present study the amino acid availabilities estimated correspond only to apparent availabilities. Wilson *et al.* (1981) found very little difference between true and apparent availabilities of amino acids of a number of test ingredients in channel catfish, *Ictalurus punctatus* (Rafinesque) and recommended that true amino acid availabilities need to be estimated only in the case of ingredients with relatively low protein content. The amino acid availabilities of the diets and ingredients tested in Murray cod will therefore be of value in diet development for the species.

The TEAA, TNEAA and TAA availability of different diets have been reported to reflect their protein digestibility for other species (Wilson *et al.* 1981; Anderson *et al.* 1992; Sadiku and Jauncey 1995). With regard to Murray cod we found that the EAA availability (%) was highly correlated to PD (%), the regression being in the form of a second order polynomial given below:

$$Y = -0.546X^2 + 91.18 - 3716.75 \ (r = 0.86;\ p < 0.01)$$, where EAA availability (%), and X= PD

As indicated previously, most workers have commented on the fact that EAA availability appeared to reflect protein digestibility of diets and/or feed stuffs. On the other hand, there had been a few recent attempts to evaluate the suitability of feed ingredients, for incorporation in to fish feeds, using generalised relationships; for example, that of van der Meer and Verdegen (1996) when they found that the chemical score of an ingredient was positively correlated to the protein content. We utilised published data on PD and EAA availability of various diets and ingredients, combined with the present data and found that, as for our own data, a significant second order polynomial described the relationship between PD and EAA availability. This significant relationship, which is derived from a combination of data on different diets and feed stuffs

for different finfish species (Murray cod - present study; shortfin eel, *Anguilla australis* - De Silva *et al.* 2000; common carp, *Cyprinus carpio* L.- Hossain and Jauncey 1989; Atlantic salmon- Anderson *et al.* 1992; gilthead seabream, *Sparus aurata* L.- Lupatsch *et al.* 1997), will be of use in evaluating the EAA availability of diets and/or feed stuffs in the future. A note of caution has to be made with regard to the use of a generalised relationship as above. The above relationship does not take in to account the availability of individual amino acids. It has been reported that availability of individual amino acids become especially pronounced when protein digestibilities are low (Vens-Capell 1984). Our on going work appears (in preparation) to indicate that perhaps the degree of correlation of the EAA index of the body proteins of the fish species to that of the diet and/or the feed stuff may provide an indication of the degree of reliability of the estimations of EAA availability using the general relationship described here.

4.4.3 Experiment 3. Changes in amino acid and fatty acid composition of developing eggs and larvae, of Murray cod until yolk-sac resorption

4.4.3.1 Amino acid profiles

The amount of total amino acids in eggs of Murray cod is comparable to that reported for striped mullet (Tamaru *et al.* 1992), white sturgeon (Ng and Hung 1994) and Nile tilapia (Gunasekera *et al.* 1996a), but is considerably lower than that in Atlantic salmon (Srivastava *et al.* 1995). This difference is probably a reflection of the large egg size and consequently the larger amount of yolk in the latter. Qualitatively, the predominant amino acids in the FAA pool of eggs in Murray cod were alanine, lysine, leucine and serine. This is comparable to that reported for dentex (Tulli and Tibaldi 1997), Atlantic halibut (Rønnestad *et al.* 1993) and turbot (Rønnestad *et al.* 1992), but differs from that for Atlantic salmon (Srivastava *et al.* 1995) and Nile tilapia (Gunasekera *et al.* 1996a). In Atlantic salmon and Nile tilapia the predominant amino acids in the FAA pool were aspartic acid, glutamic acid, cysteine and serine, and proline, tyrosine, histidine, valine and glycine, respectively. Therefore, it is difficult to discern a particular pattern in the FAA of fish in relation to habitat and related life history traits.

During early ontogeny, taurine (NEAA) was not detected in the FAA pool in the present study. Taurine has also not been reported in eggs of other freshwater and/or anadromous species such as rainbow trout (Zeitoun *et al.* 1977), coregonid species (Dabrowski *et al.* 1985), Atlantic salmon (Srivastava *et al.* 1995) and Nile tilapia (Gunasekera *et al.* 1996a). On the other hand, considerable amounts of taurine have been detected in the FAA pool in larvae of marine species (Rønnestad *et al.* 1992; Rønnestad *et al.* 1993; Conçeicão *et al.* 1997). Conçeicão *et al.* (1997) observed that the taurine content increased significantly during early ontogeny in turbot, and suggested that its primary role in early ontogeny may be osmolytic. It may be that taurine is less important to freshwater teleosts during early ontogeny, compared to larvae of marine teleosts. It will be of interest to investigate whether taurine appears in yolk-sac resorbed, first feeding larvae of freshwater fish when the liver becomes functional, particularly because taurine is thought to be the sole amino acid that conjugates with cholic acid to produce bile salt in teleosts (van Waarde 1988). The FAA content of fertilised eggs was about 0.19% of the total amino acid (TAA) content (of the eggs). In fish eggs the proportion of FAA in TAA is very variable (Rønnestad and Fyhn 1993). The FAA pool constituted 20-50% of the total amino acids in the pelagic fish eggs but in the case of marine demersal eggs this was only about 2-3% (Rønnestad and Fyhn 1993). In eggs of freshwater fish the proportion of FAA in the TAA pool varied between 4.4 and 4.7% in Coregonid fishes (Dabrowski *et al.* 1985), 2% in diadromous Atlantic salmon (Srivastava *et al.* 1995) and 0.53% in Nile tilapia (Gunasekera *et al.* 1996a; 1996b).

Unlike in the case of marine fish species, the role of FAA in early development is relatively less understood in freshwater species. The proportion of FAA in the TAA pool, as well as the absolute amount of FAA in an individual, increased significantly during development. Comparable observations have been reported for Atlantic salmon (Srivastava *et al.* 1995). In a study on rainbow trout eggs it was reported that free amino acid concentrations declined just after fertilisation, increased to near initial values in the blastula stage and then nearly doubled at hatching (Zeitoun *et al.* 1977). Terjesen *et al.* (1997) reported that during early ontogeny in African catfish (*Clarias gariepinus*) the protein content in the egg decreased and the FAA content increased until hatching, as in the case of Murray cod. On the other hand, in marine species that lay pelagic eggs, such as for example, cod (Fyhn and Serigstad 1987) Atlantic halibut (Rønnestad *et al.* 1993), and turbot (Rønnestad *et al.* 1992) the FAA content decreased with ontogeny.

4.4.3.2 Fatty acid profiles

With regard to fatty acids, in relation to early development in fish, apart from a few exceptions (Wiegand 1996; Desvilettes *et al.* 1997a), the great majority of studies have been done on marine species. In Murray cod, PUFA (both n-3 and n-6) was the predominant group of fatty acids in total lipid, and accounted for over 50% of the fatty acids in the total lipid in all the developmental stages investigated. Predominance of PUFA in total lipid in eggs and larvae has also been reported for chinook salmon (Ashton *et al.* 1992), Atlantic halibut (Falk-Petersen *et al.* 1986), whitefish (Soivio *et al.* 1989) and pike (Desvilettes *et al.* 1997a). On the other hand, for species in which lipids are utilised as the major energy source during ontogeny, saturates and monoenes have been found to be the predominant group of fatty acids such as in Senegal sole (Vázquez *et al.* 1994) and striped bass (Chu and Ozkizilcik 1995; Harrell and Woods 1995), respectively. The n-3 to n-6 ratio in Murray cod during developmental stages is typical of freshwater fish (Henderson and Tocher 1987).

With regard to the fatty acid profiles of eggs and larvae of fish, there are conflicting observations in respect of those which occur in small amounts (<0.1% of total lipid). For example, in Murray cod, in all the developmental stages investigated, 22:4(n-6) was found in significant amounts, and 22:5(n-6) was detected only in trace amounts. This observation is in agreement with that of Anderson and Arthington (1989) on the eggs of six Australian fish species (including Murray cod). On the other hand, 22:5(n-6) has not been reported in all species investigated. For example, 22:5(n-6) was reported in eggs and larvae of Atlantic halibut (Bruce *et al.* 1993) and Senegal sole (Mourente and Vázquez 1996), but not in Atlantic cod (Fraser *et al.* 1988), turbot (Silversand *et al.* 1996) and pike (Desvilettes *et al.* 1997a). Similarly, 18:3(n-6) has not always been reported in eggs and larvae of fish, and when present it has accounted for less than 0.5% of fatty acids in total lipid (Fraser *et al.* 1988; Bruce *et al.* 1993). Observations on 20:3(n-3) and 20:4(n-3) are also conflicting, the former being reported in Senegal sole (Mourente and Vázquez 1996), turbot (Silversand *et al.* 1996) and in Murray cod. These fatty acids however, were not found in Atlantic cod (Ulvund and Grahl-Nielsen 1988) and in a previous study on six Australian fish species (Anderson and Arthington 1989).

In most fish species, the fatty acids that are found in highest amounts are generally 16:0, as observed in Atlantic herring (Tocher *et al.* 1985) and Senegal sole (Vázquez *et al.* 1994), 18:1(n-9) as observed in chinook salmon (Ashton *et al.* 1992), and striped bass (Chu and Ozkizilcik 1995; Harrell and Woods 1995), and 22:6(n-3) as in almost all other species that have been investigated, including Murray cod. However, it is interesting to note that the proportion of 20:4(n-6) in total lipid in eggs and developing larvae of Murray cod was considerably higher than that reported for other fish species. One reason for this may be the routine feeding of broodstock with ox liver for a period of two months prior to spawning, as part of a conditioning diet.

Furthermore, as Murray cod development proceeded the amount of 20:4(n-6) in total lipid increased significantly. This may suggest that broodstock diet may not necessarily be the only factor responsible for an elevated level of 20:4(n-6). Such increases were concomitant with decreases in 18:2(n-6) and 20:3(n-6) but not significantly. This may be indicative of biosynthesis of 20:4(n-6) during early ontogeny of Percichthyid fish. 20:4(n-6) has been shown to be a precursor for the synthesis of prostaglandins in marine fish (Sargent *et al.* 1990). The possible conservation and likely bioconversion of other homologues to 20:4(n-6) may be an indication of its essentiality in later development in Murray cod, and its possible use structurally.

It was also evident that DHA was conserved in the early ontogeny of Murray cod. According to Sargent (1995) the conclusions of which were based mostly on marine and anadromous fish, major n-3 HUFA of all fish eggs are DHA and 20:5(n-3), generally found in a ratio of approximately 2:1. However, in Murray cod the above ratio was 5.4:1 and 7.3:1, respectively, and remained almost unchanged throughout development. It has been shown that in fish, of all the n-3 PUFA, DHA has the generalised function in maintaining structural and functional integrity of cell membranes (Sargent 1995). DHA is also known to be essential for the development of the brain and the retina in marine fish (Bell and Dick 1991; Mourente and Tocher 1992). As such one would expect to encounter high levels of this fatty acid in fish tissues, as observed in Murray cod. On the other hand, in Senegal sole (Vázquez *et al.* 1994) significant reduction of DHA, 20:5(n-3) and 22:5(n-3) were observed with development. It may be that different fish species and /or species groups have different strategies in the utilisation of n-3 PUFA.

The fatty acid requirements of freshwater species, apart from a few exceptions, are known to be different from marine fish. In general, freshwater fish have an essentiality for both n-3 and n-6 PUFA, in the form of 18:3(n-3) and 18:2(n-6), whereas marine fish require C20 and C22 HUFA (Henderson and Tocher 1987;

Sargent *et al.* 1990). The fatty acid composition of eggs and associated changes in composition during development are thought to be indicative of the fatty acid requirements for growth and well being (Sargent 1995). Based on the retention of C20 and C22 HUFA of both n-3 and n-6 series by developing eggs and larvae of Murray cod it may be concluded that this species has an equally important requirement for n-6 as for n-3. Based on early studies on freshwater fish the consensus has been that freshwater fish require 18:2(n-6) and 18:3(n-3) as they have the ability to convert these to the higher homologues (Kanazawa *et al.* 1980).

In the present study there was a tendency for Murray cod to retain PUFA. Such selective retention of yolk PUFA through early development is not uncommon in freshwater fish, as observed for steelhead trout (*Oncorhynchus mykiss*) (Hayes *et al.* 1973), goldfish (*Carassius auratus*) (Wiegand 1996) and pike (Desvilettes *et al.* 1997a).

On the other hand, Henderson *et al.* (1995) demonstrated that pike was unable to synthesise 20:4(n-6) and 20:5(n-3) from 18:2(n-6) and 18:3(n-3), respectively and suggested that pike may require the former fatty acids preformed in the diet. Desvilettes *et al.* (1997b) suggested that larval pike may be able to biosynthesise 22:6(n-3) and that this trait may have developed to counteract rather unpredictable changes in abundance of microcrustacean food resources in nature, which are its main sources of C20 and C22 HUFA. First feeding larvae Murray cod (Rowland 1992) feed on microcrustaceans, and the conditions that these fish have to face in nature are possibly not that different to those of pike. It is therefore, conceivable that the hypothesis is extendable to Murray cod, a top order carnivore in Australian freshwaters, but requires further study for confirmation of the hypothesis.

In conclusion, this study supports the view that broad differences occur in the fatty acid profile, in relation to early ontogeny, in freshwater and marine fish species. The study also indicates that during early ontogeny top-order, freshwater carnivores, such as Murray cod, may have different bio-synthesising capabilities of PUFA from fish with other feeding habits.

4.4.4 Experiment 4. Performance of juvenile Murray cod fed diets with different protein to energy ratio (a study on protein sparing capabilities)

Carnivorous species generally require a high dietary protein content, and Murray cod is no exception to this rule, as the protein requirement for optimal growth of juvenile Murray cod is around 50%, by dry weight of the diet (Gunasekera *et al.* 2000). The protein sparing capabilities tend to differ amongst species, both to the extent to which protein is spared, and the energy source utilised for the sparing. For example, lipid acts as the energy source in salmonids (Hardy 2000), whilst in anguillid eels carbohydrates provides the energy source (Hidalgo *et al.* 1993; Garcia-Gallego *et al.* 1995). On the other hand, in Asian seabass, *Lates calcarifer* Bloch, (Catacutan and Coloso 1997) and hybrid tilapia (Shiau and Peng 1993) lipid is known to be spared by carbohydrates. However, the findings on protein-sparing capabilities in some warm water fish species, such as the tilapias are not uniform (El-Sayed and Garling 1988; De Silva *et al.* 1991; Hanley 1991; Shiau and Peng 1993).

In the present study the increasing dietary lipid levels did not result in a significant increase in growth rate in juvenile Murray cod, except between $P_{40}L_{10}$ and the rest, although a general trend was evident to this effect. Protein sparing does not necessarily result in better growth as shown for common dentex, *Dentex dentex* (L), sea bream, *Sparus aurata* L., and European seabass *Dicentrarchus labrax* L., but a reduction in carcass lipid (Company *et al.* 1999), in spite of high dietary lipid content, and/or a reduction in net protein utilisation (NPU) with increasing P/E ratio of diets (van der Meer *et al.* 1997), may also reflect protein sparing. In Murray cod, the carcass lipid level increased significantly in response to increasing dietary lipid level, suggesting that Murray cod is not capable of sparing protein through the utilisation of lipid. On the other hand, lipid content of body muscle did not appear to be influenced by the dietary protein content, a trend which has also been reported for other species (Regost *et al.* 2001), which was in accordance with our observation that fat deposition with increasing dietary lipid level was mostly in the peritoneum, around the gut.

On the other hand, in Murray cod juveniles, at dietary protein levels of 40 and 50%, the SGR (% day^{-1}) showed a tendency to increase with decreasing P/E ratio of the diets, albeit not significantly ($P > 0.05$). This was also the case with NPU for the 40 % protein diets. Such trends are apparent indications of protein-sparing (van der Meer *et al.* 1997). It is therefore possible that in the present study the dietary protein levels used were such that these were sufficient to satisfy the basic requirement for protein accretion, and increase

in dietary lipid resulted in increased lipid deposition. However, it was apparent that when the dietary protein level was reduced to 40 %, with a concurrent increase in lipid levels growth was not compromised. This might suggest that further reductions in dietary protein level may be possible with out compromising growth and / or carcass quality when the protein- sparing capabilities in Murray cod are better manifested.

One other factor that needs to be considered in the interpretation of the present findings is the possible influence of the level of NFE in the different diets used. As carnivorous fish have very limited capabilities of utilising complex carbohydrates (Wilson 1994), it is highly unlikely that Murray cod juveniles are capable of digesting α-cellulose and/or carboxymethylcellulose in their diets. It is also known that high dietary fibre content affects digestibility (De Silva *et al.* 1990; McGoogan and Reigh 1996), and thereby growth performance (Hilton *et al.* 1983; Lanari *et al.* 1999). On the other hand, it has been reported that in some instances growth was not affected by the dietary fibre content (Xie *et al.* 2000). In the present study if the level of NFE was the more predominant factor one would expect more pronounced and significant differences in growth related parameters and in protein accretion in Murray cod, in relation to different dietary treatments. This was not the case in the present study. Comparable findings, in which dietary lipid level has a greater influence on performance than NFE has also been reported for the European sea bass (Lanari *et al.* 1999). The latter authors also did not find any correlation between the dietary NFE content and carcass fatty acid content/ profile.

In Murray cod juveniles, the body muscle fatty acid profiles show significant differences in response to the dietary treatments. In this study, although the total lipid content of the diets was different the fatty acid composition of the diets was not altered. Therefore, the results indicate that the fatty acid composition of body muscle could be influenced by changes in the lipid content only. It was also evident that with increasing dietary lipid content there was a tendency for linoleic acid (18:2n-6) to increase at both protein levels, though not always significantly in relation to increases in dietary lipid content. Similarly, the eicosapentaenoic acid (20:5n-3) content also increased with increasing dietary lipid level for the P_{40} diets only. The docosahexaenoic acid (22: 6n-3) concentration in body muscle did not show a clear trend in relation to increasing dietary lipid content, and liniolenic acid (18:3n-3) did not appear to change at all, and the concentration of the latter remained relatively low. It is possible that the above trends reflect, indirectly, essentiality of 18:2n-6 for Murray cod.

The trend in which body muscle fatty acid profile is affected by dietary lipid level has not been reported in fish previously. However, in rainbow trout, the ration size was found to affect the muscle lipid content, which in turn resulted in a higher level of monoenes and a lower level of polyunsaturates (Johansson *et al.* 1995). It was reported recently that the dietary lipid level affected the fatty acid composition and hydrolase activities of intestinal brush border membrane in European seabass (Cahu *et al.* 2000). These authors found that the increasing dietary lipid level resulted in a lowering of n-3 PUFA and an increase in monoenes. Such a condition, concomitant with a decline in membrane enzymatic activity, has been described as a malnutrition indicator in mammals, and accordingly the authors raised the question of possible disorders of gut function occurring in fish fed high lipid diets.

The study therefore shows that the ability to spare proteins using dietary lipids Murray cod juveniles is not that clear cut as in the case for example, salmonids. In Murray cod increased dietary lipid did not result in excessive fat deposition in body muscle, but did so in the carcass. On the other hand, a reduction in the dietary protein content to 40 %, with a concurrent increase in dietary lipid did not compromise growth and/or feed utilisation. These observations suggest that further work on lipid utilisation by Murray cod is warranted.

4.4.5 Experiment 5: Growth and nutrient utilisation of Murray cod fingerlings fed diets with varying levels of soybean meal and blood meal

Juvenile Murray cod are capable of tolerating relatively high levels of SBM in the diet, but not BM. Fowler and Banks (1976) found that when FM was replaced by spray-dried BM no negative effects were observed in chinook salmon (*Oncorhynchus tshawytscha* Walbaum) up to 5% inclusion of BM. However, pathological effects were observed when FM was replaced up to 17.5% with spray-dried BM in diets. In general, the response of different fish species to BM incorporated diets have been very variable, most fish species not being able to tolerate levels exceeding 20% in the diets.

The survival of juvenile Murray cod reared on SBM dietary treatments was significantly higher ($P< 0.05$)

than those on the CD, whereas those reared on BM diets were very much lower. BM is known to contain high levels of iron (2769 mg kg^{-1}) and zinc (306 mg kg^{-1}) (National Research Council 1993). Desjardins *et al.* (1987) found that supplementing diets with ferrous sulphate caused iron catalysed lipid oxidation in the diets, increasing the malonaldehyde (MA) concentration with increasing iron level and that the effect of diet rancidity and iron overload was experienced at greater than 86 mg kg^{-1} and led to poor growth and high mortality in rainbow trout. However, when measures were taken to reduce diet lipid oxidation and rancidity (less than 10µg MA g^{-1}diet), dietary iron level toxicity was greater than 1380 mg kg^{-1} diet. In the present study, the concentrations of iron and zinc were not estimated. However, it is thought that the combination of the high levels of iron and zinc and the low digestibility of BM diets may have led to high mortalities in fish reared on BM diets, and is discussed later.

The final mean weight and SGR (% day^{-1}) of Murray cod reared on SBM dietary treatments were similar to each other and the control. The FCR, PER and PCE of all the SBM dietary treatments were also similar to the CD. This reinforces the suitability of SBM as a FM substitute in Murray cod diets, at least up to the maximum level tested presently. Instances in which the SBM diets have performed better than FM control diets, as in the present study are known (Webster *et al.* 2000), and it is possible that such effects result from relatively poor quality of FM.

SBM is a commonly used ingredient in most fish feeds, and the amount of SBM that could be included without compromising survival, growth and carcass quality tend to vary amongst cultured fish species, and even amongst closely related species. For example, the Australian shortfin eel (*Anguilla australis* Richardson) is known to be capable of tolerating higher amounts of SBM in the diets compared to the closely related European and Japanese eels (De Silva *et al.* 2001), as is the case with rainbow trout and Atlantic salmon (Refstie *et al.* 2000). Furthermore, up to 80 % of the FM could be replaced with SBM in channel catfish (*Ictalurus punctatus* Rafinesque) (Robinson 1992) and hybrid striped bass diets (Gallagher 1994). However, complete replacement with soy protein concentrate in rainbow trout diets has yielded contradictory results (Kaushik *et al.* 1995; Stickney *et al.* 1996).

The amount of SBM that could be used in fish diets is often limited by amino acid imbalances, especially limitation of sulphur amino acids, and the presence of anti-nutritional factors such as protease inhibitors. In the present study however, any anti-nutritional factor contained in the defatted SBM used did not cause any noticeable adverse effects on growth or feed utilisation etc. at the levels tested. A study by Boonyaratapalin *et al.* (1998) on solvent extracted-, extruded full-fat-, steamed full-fat- and soaked raw full-fat- SBM at 15 % of the dietary protein against a FM control diet in Asian seabass (*Lates calcarifer* Bloch) found that growth was significantly reduced using all but the solvent extracted SBM diet. In a similar study on full-fat-, full-fat extruded-, solvent extracted-, and solvent extracted and infra-red radiation treated- SBM diets at 30 % of the dietary protein against a brown FM control diet in rainbow trout indicated that only the diet containing full-fat extruded SBM reduced growth significantly (Oliva-Teles *et al.* 1994). In the present study no reduction in growth or feed utilization was observed in all of the diets containing SBM when compared to the control. This suggests that the upper level to which SBM can be higher than the highest level tested presently.

The % ADMD and % PD, of all SBM diets were similar to that of the control, and corresponded to the results on growth and feed utilisation of these diets. The % ADMD and % PD of the BM diets, on the other hand, tended to decrease with increasing level of BM inclusion and corresponded with the ingredient digestibility results (data not presented), which supports the observations of poorer growth and feed utilisation seen with increasing level of inclusion. Our observation that faecal matter had a powdery consistency, with black fine particles, thought to be BM in the feed, indicates that Murray cod did not digest these diets well. This is also consistent with the digestibility estimations, as well as the result that the CoV of both ADMD and PD estimations increased significantly with increasing inclusion of BM in the diets.

Most carnivorous fish, including Murray cod, are known to digest SBM rather well (Wilson *et al.* 1981; Hajen *et al.* 1993; Lupatsch *et al.* 1997; Bransden and Carter 1999; De Silva *et al.* 2000). On the other hand, ADM and PD of BM diets by rainbow trout and Atlantic salmon are known to be extremely variable (see Hajen *et al.* 1993), and so is the case of carnivorous species generally (da Silva and Oliva-Teles 1998). But more recently Bureau *et al.* (1999) reported that spray-dried BM to be highly digestible in rainbow trout. Wang and Parsons (1998) found that in diets for poultry, raw material source, processing temperature and cooking time affected the ingredient amino acid digestibility of meat and BM. In the present study the BM used was a commercial preparation, and purported to have been spray dried. It is possible that one or a combination of

these factors may have resulted in the low digestibility of BM diets in the present study.

In conclusion, the study indicates that Murray cod, a top order carnivore, can be reared on diets in which up to 32 % of the FM protein is replaced by SBM, without compromising survival and/ or growth performance. Inclusion of relatively high level of SBM in Murray cod diets will result in significant reductions in feed costs, and therefore will contribute to making the industry economically feasible.

4.4.6 Experiment 6: Evaluation of experimental diets under commercial scale

The protein (Gunasekera *et al.* 2000) and lipid requirements (De Silva *et al.* 2002) of Murray cod are known, but not the amino acid and fatty acid requirements. However, the amino acid contents of the test diets conformed to the amino acid index of Murray cod eggs and larvae (Gunasekera *et al.* 1999a) and muscle (Gunasekera *et al.* 2002). The fatty acid profile of the diets matched those of carnivorous fresh water fish (National Research Council 1993), as well as that of Murray cod eggs and larvae (Gunasekera *et al.* 1999a; 1999b). As such it is thought the diets used were nutritionally adequate for growth and well being of Murray cod under aquaculture conditions. Therefore, any differences in the performance of Murray cod reared on different diets in the current trials are not thought to result from any nutritional inadequacies, but differences in diet palatability, digestibility and possible nutritional imbalances (eg. excess lipid content; imbalance in n- 3 to n- 6 ratio etc.).

The growth rates and feed utilisation indicators observed for the four test diets were good, and a lack of significant differences amongst diets for most of the parameters tested may be indicative of the suitability of all the diets for Murray cod culture. However, the feed intake, FCR and PER of fish fed DU1 and DU2 diets were consistently better than the corresponding commercial diets tested, and indicates better utilisation efficiencies of the former diets by Murray cod. Murray cod has shown to be incapable of sparing proteins (De Silva *et al.* 2002), and hence the relatively high lipid content in the two commercial diets could have lead to higher carcass lipid deposition, as well as higher HSI.

The DU2 was the least expensive, because of the inclusion of about 20% SBM, which is known to be well digested by Murray cod (De Silva *et al.* 2000). Abery *et al.* (2002) demonstrated that SBM could be substituted by up to 32% in diets for young Murray cod without compromising growth performance and/or carcass quality. The present observations complement the earlier findings, and suggest that commercial diets for Murray containing at least up to 20% if not 32% SBM could be used in Murray cod culture. It was observed that the DU2 diet however, was less water stable, and tended to break faster. This is not considered a major problem, as the addition of a low cost binder could overcome this deficiency.

It is evident that overall, the fatty acid profile of muscle of Murray cod reflected that of the diets, not entirely, however. For example, the amount of monoenes in muscle lipid of Murray cod tended to be significantly lower that that of the corresponding diet, and also the amount of HUFA tended to be regulated. These observations are comparable to those reported on sharpsnout sea bream (Rueda *et al.* 2001), and provided support for the proposition (Rueda *et al.* 2001) that fish may be endowed with a mechanism that limits the accumulation of specific fatty acids in the tissues irrespective of dietary availability. All the diets tested had higher amounts of n- 3 PUFA than n- 6 PUFA, and consequently resulted in relatively high and a uniform n- 3 to n- 6 ratio in muscle, almost four times higher than that in wild Murray cod. Bell *et al.* (1998) reported that farmed Atlantic salmon had a lower n- 3 to n- 6 ratio than in wild counterparts, and the difference was attributed to use of low levels of vegetable oils, which elevated the 18:2n- 6 levels in the feeds. Unlike in the case of Atlantic salmon the balance of previous evidence has indicated (Gunasekera *et al.* 1999b; Turchini *et al.* 2003) that Murray cod has a requirement for n- 6 series fatty acids, over n- 3. However, the present trials indicate that when these requirements are satisfied, the feeds can be developed to increase the n- 3 level in muscle, as desired for human health reasons (Stansby 1990; Kelly 1991; Ulbricht and Southgate 1991).

4.4.7 Experiment 7: Comparisons of the fillet quality of cultured and wild Murray cod

The lipid content of muscle of farmed and wild Murray cod was less than 2.0 %, and as such, it can be considered to be a non- fatty fish. Interestingly, Murray cod even when fed diets with relatively high lipid content do not appear to deposit fat in muscle in a proportionate manner to the dietary lipid content. On the other hand, the growth trials indicate that carcass lipid content could be as high as 8.9 %, suggesting that excess dietary lipid is mostly deposited as visceral fat in Murray cod. The location of additional fat deposition in fish depends on species and size (Sheridan 1998).

The most marked difference between the two groups was in the amount of 20: 4n- 6, which in wild Murray cod was about five to six times higher than in the farmed fish, an observation which has also been reported for sharpsnout sea bream (Rueda *et al.* 2001) and other Mediterranean species (Zlatanos and Sagredos 1993). In general, more attention has been given in recent years to comparisons of fatty acid profiles of wild and farmed fish, such as Atlantic salmon (Bergström 1989), red drum (Villarreal and Rosenblum 1994), red porgy (Rueda *et al.* 1997), turbot (Sérot *et al.* 1998) and sweet smelt (Jeong *et al.* 2000). The most significant differences in wild and farmed Murray cod were observed in respect of the fatty acid profiles. In wild Murray cod the n- 6 series fatty acids (by virtue of having relatively high amounts of 20: 4n- 6), without exception, tended to be significantly higher than in farmed fish, where as the reverse was true for the n- 3 fatty acids, with the exception of linolenic acid (18: 3n- 3) which was higher in wild fish but not significantly so. The above differences are exacerbated in the significantly lower n- 3 to n- 6 ratio in wild Murray cod (1.0 vs 5.2). Such uniform differences in the fatty acid composition of wild and farmed fish have not been reported hitherto. For example, in European eel higher levels of HUFA were observed in farmed fish (Abrami *et al.* 1992); in red drum 18:2n-6 (linoleic acid) was lower in wild fish whilst 20:4n- 6 (arachidonic acid) and 22:4n- 6 (adrenic acid) were higher in wild fish, and so was 22:5n- 6 (Villarreal and Rosenblum 1994); farmed turbot contained lower proportions of long chain n- 3 PUFA and 20:4n- 6, but higher proportion of 20:1 and 22:1 and the n- 3: n- 6 ratio was considerably higher in wild fish (8.8 vs 4.2; Sérot *et al.* 1998); in red porgy both n- 3 and n- 6 fatty acids were found in higher amounts (Rueda *et al.* 1997), and in farmed Atlantic salmon 18:2n- 6 was higher than in wild fish, and consequently the n- 3 to n- 6 ratio in the former ranged from 3.2- 6.9, much less than in wild fish (10.3; Bell *et al.* 1998).

Fatty acid composition is affected by a number of factors, the foremost of these is the diet. In Murray cod, the fatty acid profile of wild and farmed fish indicate that the species has a tendency to conserve n- 6 PUFA in preference to n- 3 HUFA, whilst retaining the total PUFA and HUFA levels unchanged. This trend is also evident from studies on the early ontogeny of the species (Gunasekera *et al.* 1998).

The atherogenicity and thrombogenicity indices of Murray cod reared under different dietary treatments as well as in farmed and wild fish analysed were not widely different in the different groups. These indices take in to account the relative proportions of saturates, monoenes and PUFAs, and the known roles of individual fatty acids in reducing the risks of artherosclerosis and thrombosis (Ulbricht and Southgate 1991). Both indices for Murray cod were comparable to that of raw mackerel, which is considered as a baseline.

Previous studies have indicated that Murray cod has a requirement for n-6 PUFA (Gunasekera *et al.* 1999b), and provides further evidence in this regard by the fact the amount of n-6 in muscle of wild Murray cod is considerably higher than that of n-3 PUFA, resulting in a very low n-3 to n-6 ratio of about 1.0. This ratio is amongst the lowest recorded for freshwater species (Henderson and Tocher 1987). Murray cod is a top-order predatory carnivore and in the wild feeds on a range of aquatic animals including crustaceans, fish, reptiles, frogs and water birds (Harris and Rowland 1996). Fresh water fish (Henderson and Tocher 1987; De Silva *et al.* 1998) are known to contain much less n-3 PUFA. More recently, laboratory findings on Murray cod nutrition however, indicate that it has specific n-3 to n-6 requirements for optimal growth and feed utilisation, in the range of 1.2:1.0 (Turchini *et al.* 2003). Also, the current growth trials indicated that the fatty acid content of muscle reflects that of the feed, and that Murray cod feed could be formulated in such a manner as to satisfy consumer acceptability or to increase the n-3 levels with out compromising growth performance.

4.5 General Conclusions

- Murray cod being a top order carnivore, and as expected, requires a dietary protein content of about 50 %, by dry weight, for optimal growth performance.
- The study on fatty acid and amino acid profiles during early ontogeny suggest that C20 and C22 HUFA of both n-3 and n-6 series are retained by developing eggs and larvae of Murray cod, and as such it may be concluded that this species has an equally important requirement for n-6 as for n-3 possibly in the form of the base fatty acids 18:2(n-6) and 18:3(n-3).
- Murray cod is able to digest (dry matter, nutrients and EAA) food industry by-products such as defatted SBM, SMM and MM effectively.
- The "protein sparing" abilities of Murray cod, through lipid as an energy sources are relatively limited. Indeed, when Murray cod is fed diets containing in excess of 17% lipid, growth performance is affected

and the fish tend to deposit excessive fat in the peritoneum, but not necessarily in the muscle. This excessive deposition of fat could influence its marketability.

- In view of the above, the avenues available for cost-reduction in Murray cod feeds need to be explored through FM replacement, through suitable protein rich ingredients, either partially or wholly, rather than through high energy diets. In this regard the experiments indicate that up to 32% of the FM content in Murray cod diets could be replaced without compromising growth performance, food conversion efficiency, protein efficiency ratio and net protein utilisation, and carcass quality.
- As such, a standard diet has been formulated, and improved upon, and is being tested under a commercial setting currently.
- Since the fatty acid content of Murray cod muscle reflects that of the diet, feeds could be formulated in such a manner as to satisfy consumer acceptability or to increase the n-3 levels with out compromising growth performance.
- The current study, together with previous findings, provides sufficient information to develop relatively cost- effective commercial diets for the intensive culture of Murray cod. Successful testing under commercial conditions of the DU2 diet in particular is a significant development to further expansion of the Murray cod farming industry. This diet has better performance characteristics and is more cost-effective than the "off-the-shelf" diet commonly used by the industry currently. The current study showed that use of the DU2 diet could substantially reduced feed costs by as much as $AUD 490-500 tonne^{-1} of fish produced. Feed costs typically account for about 20-30% of costs of producing Murray cod in intensive recirculation systems (Rawlinson 2004). Adoption of the DU2 diet for grow-out of Murray cod in these systems will significantly reduce the overall cost of production.

4.6 Acknowledgments

Funding for this project was provided by the Fisheries Research and Development Corporation (Project No. 1999/328), Fisheries Victoria (Department of Natural Resources and Environment) and Deakin University. The authors wish to thank the kind support and assistance of staff at Primary Industries Research Victoria, Snobs Creek, Australian Aquaculture Products Pty Ltd, Euroa, including Roger Camm, Steve Vidler and Lynton Fisher, and Peter and Carla van Lierop of the Alexandra Fish Farm, Alexandra.

4.7 Abbreviations

ADM	Apparent dry matter
ADMD	Apparent dry matter digestibility
AI	Atherogenicity index
BM	Blood meal
CD	Control diet
CD/B	Commercial diet / barramundi
CD/S	Commercial diet / salmon
CMC	Carboxy methyl cellulose
DHA	Docosahexaenoic acid
EAA	Essential amino acids
ED	Energy digestibility
EFA	Essential fatty acids
FAA	Free amino acids
FEAA	Free essential amino acids
FMOC	9-fluroenylmethyl chloroformate
FNEAA	Free non-essential amino acids
FCR	Food conversion ratio
FE	Fertilised eggs
FM	Fish meal
HSI	Hepatosomatic Index
HUFA	Highly unsaturated fatty acids
MM	Meat meal
MUFA	Monounsaturated fatty acids
NEAA	Non-essential amino acids
NFE	Nitrogen-free extract
NHL	Newly hatched larvae
NPU	Net protein utilisation
PD	Protein digestibility
PCE	Protein conversion efficiency
PER	Protein efficiency ratio
PUFA	Polyunsaturated fatty acids
RD	Reference diet
SBM	Soybean meal
SGR	Specific growth rate
SBM	Soybean meal
SMM	Shark meat meal
TAA	Total amino acids
TNEAA	Total non-essential amino acids
TEAA	Total essential amino acids
TI	Thrombogenicity Index
TL	Total length
WF	Wheat flour
YSRL	yolk-sac resorbed larvae

4.8 References

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5 WATER QUALITY AND INTENSIVE MURRAY COD AQUACULTURE

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Boreham, S., Ingram, B.A., Jones, P.L. and Collins, R.O. (2004). Water quality and intensive Murray cod aquaculture. In: *Development of Intensive Commercial Aquaculture Production Technology for Murray cod* (ed. B.A. Ingram and S.S. De Silva), pp 113-128. Final Report to the Fisheries Research and Development Corporation (Project No. 1999/328). Primary Industries Research Victoria, DPI, Alexandra, Victoria, Australia.

CONTENTS

5.1 Introduction

A major proportion of finfish aquaculture currently occurs in open, extensive/semi-intensive pond or cage culture systems. However, these systems can have detrimental impacts on the surrounding environment by, for example, output of waste products, (nutrients, suspended solids, animal wastes, chemicals etc), consumption and degradation of water resources and escape of stock (and associated risk of disease transmission and genetic contamination) (Pillay 1992; Baird *et al.* 1996).

With increasing emphasis on environmentally/ecologically sustainable development of food production methods, there is an increasing trend towards the use of more intensive production systems, such as intensive recirculating aquaculture systems (RAS). A standard feature of these systems is that a substantial proportion of the water utilised in the culture units (mostly tanks) is passed through a water treatment system and reused, which saves both water and power for heating and cooling. Advantages of RAS include compactness (small footprint), biosecurity, high density production and their ability to control the environment in which the animals are reared. RAS are generally more environmentally friendly than other types of aquaculture systems due primarily to their reduced water usage and control waste discharge.

Forms of water treatment in RAS may include physical, chemical and biological filtration, oxygen injection and some forms of pathogen control, generally via ultra violet light (U.V), chemical or ozone treatment. Methods of solids removal may come in the form of rotating drum-screen filters or sand filters for removal of suspended solids. Bio-filtration may come in the form of rotating biological contactors, fluidised bed filters, bead filters or trickle filters.

The Murray cod (*Maccullochella peelii peelii*) aquaculture industry is growing in Australia. While the

fingerling production sector is well established and based on extensive culture of juveniles in open earthen ponds, the table fish production sector is in a developmental phase with production levels increasing significantly over the past five years (Ingram and Lawson 2004). To date, the grow-out of Murray cod occurs predominantly in RAS. However, very little information is available on the specific water quality requirements for optimal production of Murray cod under commercial conditions. Maintenance of broodfish and rearing of juveniles in earthen ponds have provided some basic information on water quality under which the fish will survive and grow, but although Murray cod are now being reared in RAS, no information is available on the water quality conditions under which these fish are exposed. In the absence of this information, ranges of parameters published for other warmwater species have been used as a guide for Murray cod aquaculture (Ingram and Larkin 2000).

In the freshwater environment, nutrients (primarily nitrogen (N) and phosphorus (P)), suspended solids, dissolved oxygen and biochemical oxygen demand are of particular environmental and regulatory interest. Phosphorus is often considered to be the major nutrient limiting algal growth and eutrophication in natural freshwater systems. Dissolved (soluble) P directly affects water quality and is immediately available as a nutrient for plant growth. Freshwater aquaculture operations are often considered as a source of these nutrients and as such may contribute to eutrophication of receiving waters (Pillay 1992; Rosenthal 1994). In Victoria, for example, trout farming in the Goulburn-Broken River catchment has been reported to contribute up to 29 t/annum of total P to the catchment which represented 8% of total catchment loading in 1993/94 (GBWQWG 1995; Ingram 1999b). To date there have been no attempts to determine the amounts of nutrients discharged, and associated environmental impacts, from intensive RAS used to culture Murray cod.

5.1.1 Objectives

The objectives of this study were to:

- Investigate water quality within an intensive Murray cod RAS and, in particular, examine how certain physical and chemical parameters change as the water passes through the various components of the water treatment plant within the RAS.
- Assess the level of nutrients (N and P) discharged from an intensive Murray cod RAS operation through the use of a conceptual nutrient mass balance model.
- Review the water quality requirements for production of Murray cod under commercial conditions.

5.2 Materials and methods

5.2.1 Australian Aquaculture Systems Pty Ltd. (AAS)

The recirculation system evaluated is a commercial facility growing Murray cod located in Alexandra, Victoria, belonging to Australian Aquaculture Systems Pty Ltd. The system has a total water capacity of 172 m^3, of which 122 m^3 is in 15 circular tanks (2-15 m^3 capacity) and the balance is in pipes and the water treatment plant. Approximately 20% of the water within the system is replaced daily by water from the Alexandra domestic water supply. Fish are cultured densities of about 140 - 180 kg fish/m^3. The system is rated at 25 tonne production capacity per year.

Fig. 5.1 describes where important treatment components are located and outlines the general direction of water flow. The three main water treatment components in the system are the drum filter, submerged upflow filter and trickle filter.

5.2.1.1 Mechanical filtration

Primary solids removal is undertaken by a Hydrotech drum filter fitted with a 40 μm screen. This is also the point where most of the water is lost from the system, and is also the main effluent stream. Finer suspended solid particles (< 40 μm) are removed by the submerged up-flow filter, a 7.6 m^3 cylindrical tank containing Bionet® 200 (specific surface area of 200 m^2/m^3). This filter is backwashed for about one hour once per week and is the second effluent stream from the systems. Each tank is also fitted with a screen on the outlet pipeline. Uneaten food that accumulates against these screens is removed by hand daily.

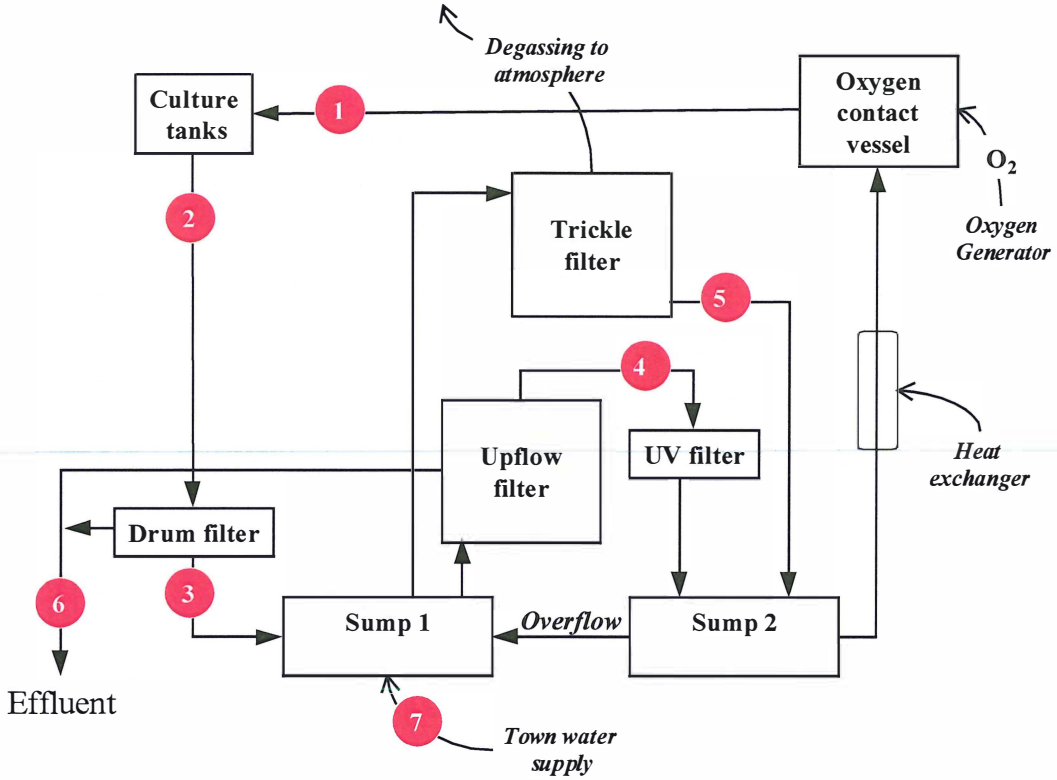

Fig. 5.1. Simple schematic of the AAS recirculating aquaculture system showing key components, flow of water (arrows) and sampling points within the system. (1) Pre culture tank, (2) Post culture tank, (3) Post drum filter, (4) Post upflow filter, (5) Post trickle filter, (6) Drum filter effluent and (7) Inlet water (town water supply).

5.2.1.2 Biological filtration

The trickle filter, contains 48 m^3 unit of Bionet® 200 (total surface area 9,600 m^2) and is the principal biofilter withinin the system, being responsible for converting most of the metabolically derived soluble nitrogen containing waste products. Water flow through the trickle filter was approximately 150,000 L/hr. An exhaust fan situated above the trickle filter facilitated the degassing of carbon dioxide (CO_2) from the water within the trickle filter.

5.2.2 Sampling

Sampling was undertaken over a 13 week period commencing on 10 September 2001. Water samples, which were taken on Mondays and Thursdays at 09:00 hrs, were collected from six sampling points within the system (Fig. 5.1). These were:

- Pre culture tank, after oxygenation (via an oxygen contact vessel).
- Post culture tank, prior to entering the drum filter.
- Post drum filter
- Post upflow filter
- Post trickle filter
- Drum filter effluent
- Inlet water (town water supply).

5.2.3 Water Quality Analysis

Dissolved oxygen (DO), temperature and pH were measured *in situ* by portable water quality meter (Yellow Springs Instruments and Hanna). For other parameters, approximately 500 mL of water was collected in acid-washed glass jars and returned to the laboratory on ice for subsequent analysis.

Total ammonia- nitrogen (TAN) (NH_3–N), nitrite- nitrogen (NO_2-N), nitrate- nitrogen (NO_3-N), total phosphorus, turbidity, carbon dioxide (CO_2) (on 3 occasions only) were measured with using Hach procedures on a Hach DR 4000 (or a Hach DR2000, some parameters during diurnal sampling only). Total alkalinity was determined by titration. Biochemical oxygen demand (BOD_5) (measured weekly only) was determined using "WTW Oxitops " incubated at 20°C for five days. Unionised ammonia (UIA) was determined from formulae provided by Emerson *et al.* (1975).

Suspended solids was determined by filtering 50mL of water through a pre-weighed Whatman GF-C filter paper, and measuring change in weight of the filter paper following drying (APHA 1992). Total dissolved solids were analysed using the supernatant of the suspended solids samples by firstly weighing a clean and dried porcelain evaporating dish, then slowly evaporating 50mL of the sample over a stovetop until dry, cooling in a desiccator and re-weighing.

5.2.4 Effluent discharge

Two models were used to estimate the amounts of nutrients (N and P) discharged for every tonne of Murray cod produced in a RAS. These models were:

- *Simplified nutrient mass balance model* (Model A) was used to derive levels of P and N discharged from the RAS. This model, which was based on models for trout farming in Victoria presented by GBWQWG (1995) and Ingram (1999b), uses estimates N and P in feed and fish, and FCR's to estimate amounts of N and P discharged.
- *Simplified water budget* (Model B) estimated the amount of P discharged from estimates of P in the effluent water (drum filter effluent) measured during the present study and the volume of water discharged from the RAS annually. An estimation of N discharged was not undertaken by this method, as total N concentrations in the effluent water were not determined during the present study.

5.2.5 Statistical analysis

To determine effects of fish biomass in the culture tanks and the individual filtration components (drum filter, upflow and trickle filter) on water quality, statistical analysis was preformed using a paired t-test. Significant differences were deemed to have occurred when $p < 0.05$.

5.3 Results and Discussion

5.3.1 Water quality

The water quality in the system varied significantly for all parameters over the period of sampling. Summary data (minimum-maximum (mean)) for all parameters measured at each point in the RAS are presented in Table 5.1.

5.3.1.1 Temperature

Temperature ranged from 17 – 22.8°C (19.8°C). Temperature did not differ greatly between sample points over the duration of the trial. However, temperature increased during the sampling period, which was attributed to increasing ambient temperatures associated with the onset of summer. Temperature was also affected by the weekly backwashing of the upflow filter. In the 24 hours following a backwashing event, temperature immediately declined by approximately 0.2°C, but recovered to pre backwash levels within 24 hours. This drop in temperature was attributed to a dilution of the system water by considerably colder town water, that had an average temperature of 13.9°C (Table 5.1).

5.3.1.2 Dissolved oxygen (DO)

Not surprisingly, DO concentrations in pre culture tank samples (mean 15.9 mg/L, 170% saturation) were significantly ($P = 0.001$) and constantly greater than concentrations in the post culture tank samples (8.5 mg/L, 92% saturation) (Table 5.1, Fig. 5.3a), and all other sampling sites within the system (Fig. 5.3a). High

DO concentrations in the pre-culture tank water were due to direct oxygen injection via an oxygen contact vessel into the water (approximately 1.6 kg/hr O_2) (Fig. 5.1), whereas concentrations in the post culture tank was reduced by consumption of oxygen by fish in the culture tank. DO concentrations in the water increased significantly (P = 0.0001) after passing through the drum filter, but were significantly (P = 0.0001) decreased by both the upflow filter and the trickle filter (Fig. 5.3). These latter trends may have been due to the process of nitrification consuming oxygen within the upflow filter and the trickle filter. The lowest DO concentrations (mean 5.7 mg/L, 61.5% saturation) were recorded in the drum filter effluent, presumably due to the high suspended solids content and the subsequent high average BOD of 45.6mg/L (Table 5.1).

5.3.1.3 pH

pH, which ranged from 5.4 – 6.8 (mean 6.1), showed variation throughout the period of sampling. Generally, pH was significantly (P = 0.0001) higher in the pre culture tank water than the post-culture tank water (Fig. 5.2). The lowest pH readings (minimum 5.4) were recorded in the post upflow filter water. pH readings were significantly (P = 0.0001) greater following passage through the trickle filter (Fig. 5.3b). This trend may have been associated with degassing of CO_2 within the trickle filter. pH within the system was buffered by regular addition of sodium hydroxide (approximately 12 kg/wk), with the aim to maintain a reading of about 6.0 and 6.5.

5.3.1.4 Ammonia (TAN and UIA)

TAN concentrations varied considerably over the period of the trial, ranging from 20-37 mg/L (mean 30 mg/L). TAN concentrations were significantly (P=0.004) higher in post-culture tank samples than pre-culture tank samples (Fig. 5.3c). The drum filter did not significantly (P = 0.41) effect TAN concentrations. However, TAN concentration were significantly (P < 0.004) reduced in water samples following passage through both the upflow filter and the trickle filter, indicating that some nitrification was occurring within these filters (Fig. 5.3c). Highest TAN concentrations (23-51 mg/L; mean 36 mg/L) were observed in the drum filter effluent whereas concentrations in the town water supply were below the level of detection (Fig 5.3c).

Unionised ammonia (UIA) showed a sharp peak of 0.09 mg/L on the 1st October 2001, which corresponded with a peak in both temperature and TAN. Apart from this one peak, UIA remained below 0.04 mg/L throughout the sampling period (Fig 5.2c). No studies have been undertaken to determine the effects of UIA on Murray cod. However, the threshold UIA concentration above which the growth of juvenile silver perch is reduced by 5% was estimated to be 0.06 mg/L (Frances *et al.* 2000). For the most part, UIA concentrations in the present study were below this threshold. UIA concentrations were higher in the pre-culture tank water than the post culture tank water (Fig. 5.2c), which may have been due to the higher pH in the former affecting the proportion of TAN that is in the unionised form (Emerson *et al.* 1975).

5.3.1.5 Nitrite (NO_2-N) and Nitrate (NO_3-N)

NO_2-N concentrations, which ranged 0.03 to 1.22 (mean 0.32 mg/L), varied little between the different components of the RAS. NO_3-N concentrations, were highly variable, ranging from 7-95 mg/L (mean 46 mg/L) within the system. Passage of water through the drum filter, the upflow filter and the trickle filter did not significantly (P > 0.05) effect concentrations of either NO_2-N or NO_3-N. No studies have been undertaken to determine the effects of NO_2-N and NO_3-N on Murray cod. However, Frances *et al.* (1998) found that the growth of silver perch was affected only after NO_2-N exceeded 1.43 mg/L. Highest NO_2-N concentrations (0.21 – 1.78 mg/L (mean 0.68 mg/L) were observed in the drum filter effluent (Table 5.1). NO_3-N concentrations in RAS often exceed 100 mg/L (Hrubec *et al.* 1996). Although nitrate is generally considered non-toxic to fishes, Hrubec *et al.* (1996) suggested that prolonged exposure to elevated levels may stress fish, affect the immune response and lead to increased mortalities.

5.3.1.6 Phosphorus (P)

Total P concentrations ranged from 2.7 – 42.5 mg/L (mean 12.8 mg/L) within the system during the present study. Neither the drum filter, upflow filter nor trickle filter significantly affected total P concentrations in water samples. Nevertheless, substantially higher total P concentrations were recorded in the drum filter effluent (mean 21.5 mg/L) than the system water (Table 5.1).

5.3.1.7 Turbidity

Turbidity, which ranged from 0-15 NTU (mean 5.8 NTU), was greatest in the post-culture tank water samples and lowest in the post upflow filter samples (Fig. 5.3 d). Turbidity was significantly increased following passage through the culture tanks, but was significantly reduced by both the drum filter and the upflow filter (Fig. 5.3d). Turbidity was not significantly affected by passage through the trickle filter.

Table 5.1. Summary water quality data (range (mean)) recorded from a Murray cod RAS during the present study

Parameter	Pre culture tank	Post culture tank	Post drum filter	Post upflow filter	Post trickle filter	System	Drum filter effluent	Town water supply
Temperature (oC)	18.1 - 23.1	18.1 - 22	18 -22.8	18.1 -22	17 -22.7	17 - 22.8	18 - 21.7	13.8 - 14
	(20.0)	(19.5)	(19.9)	(19.4)	(20.0)	(19.8)	(19.4)	(13.9)
DO (mg/L)	14.6 - 17.4	7.7 - 9.4	6.2 - 10.2	6.7 - 9.2	7.2 - 9.4	6.2 - 17.4	4.2 - 7.5	7.3
	(15.9)	(8.5)	(9.1)	(8.0)	(8.4)	(10.2)	(5.7)	
CO_2 (mg/L)	16.9 – 36.6	18.6 – 40.8	20.4 – 37.2	21.0 – 39.8	17.5 – 33.2	16.9 – 40.8	28.2 – 46.2	
	(26.8)	(29.7)	(28.8)	(30.4)	(25.4)	(28.2)	(37.2)	
pH	5.6 - 6.8	5.5 - 6.6	5.5 - 6.6	5.4 - 6.6	5.6 - 6.8	5.4 - 6.8	5.4 - 6.8	6.8 - 7.3
	(6.2)	(6.0)	(6.1)	(6.0)	(6.3)	(6.1)	(6.4)	(7.1)
TAN (mg/L)	20.2 - 36.3	22.1 - 37	21.1 - 38	20.4 -36.3	20.2 - 37	20.2 - 37	22.8 - 51	0
	(29.8)	(31.3)	(30.2)	(29.0)	(30.7)	(30.1)	(36.3)	
UIA (mg/L)	0.004 - 0.089	0.004 - 0.065	0.004 - 0.058	0.003 - 0.056	0.004 - 0.087	0.003 - 0.089	0.005 - 0.123	
	(0.025)	(0.017)	(0.016)	(0.013)	(0.028)	(0.02)	(0.044)	
NO_2-N (mg/L)	0.03 - 1.06	0.09 - 1.22	0.09 - 0.85	0.10 - 0.81	0.065 - 0.91	0.03 - 1.22	0.21 - 1.78	0.001 - 0.003
	(0.33)	(0.29)	(0.34)	(0.26)	(0.37)	(0.32)	(0.68)	(0.002)
NO_3-N (mg/L)	7 - 84	21 - 81	11 - 84	13 - 95	8 - 84	7 - 95	19 - 88	0.3 - 1.5
	(44)	(49)	(45)	(46)	(48)	(46)	(50)	(1.0)
Total P (mg/L)	3.0 - 20.1	3.9 - 23.9	6.5 - 27.4	4.5 - 42.5	2.7 - 19.4	2.7 - 42.5	7.1 - 42.5	0.05 - 0.06
	(12.2)	(13.1)	(13.1)	(13.3)	(12.5)	(12.8)	(21.5)	(0.05)
Suspended solids (mg/L)	0.4 - 21	2.2 - 54	0.2 - 35	0.4 - 8.0	0.4 - 24	0.2 - 54	26 - 719	0
	(4.7)	(11.5)	(5.6)	(2.8)	(5.2)	(5.7)	(142)	
BOD (5 day) (mg/L)	0 - 28	2 - 49	0 - 34	0 - 24	1 - 27	0 - 49	19 - 82	0
	(8.7)	(17.5)	(13.3)	(8.8)	(10.3)	(11.7)	(46.5)	
Alkalinity (mg/l)	0.4 - 27.0	1.1 - 28.5	0.9 - 21.1	0.1 - 20.5	0 - 20.6	0 - 28.5	10.8 - 120	7.2 - 13.5
	(9.4)	(9.9)	(9.1)	(7.7)	(9.6)	(9.1)	(43.8)	(9.4)
Turbidity (NTU)	0 - 15	4 - 33	2 - 12	0 - 11	1 - 13	0 - 15	7 - 1343	0
	(5.2)	(10.1)	(5.3)	(3.9)	(5.4)	(5.8)	(173.5)	
Dissolved solids (mg/L)	44 - 1108	48 - 1164	46 - 1230	44 - 1024	44 - 1216	44 - 1230	52 - 1344	15
	(356)	(352)	(370)	(320)	(357)	(351)	(402)	

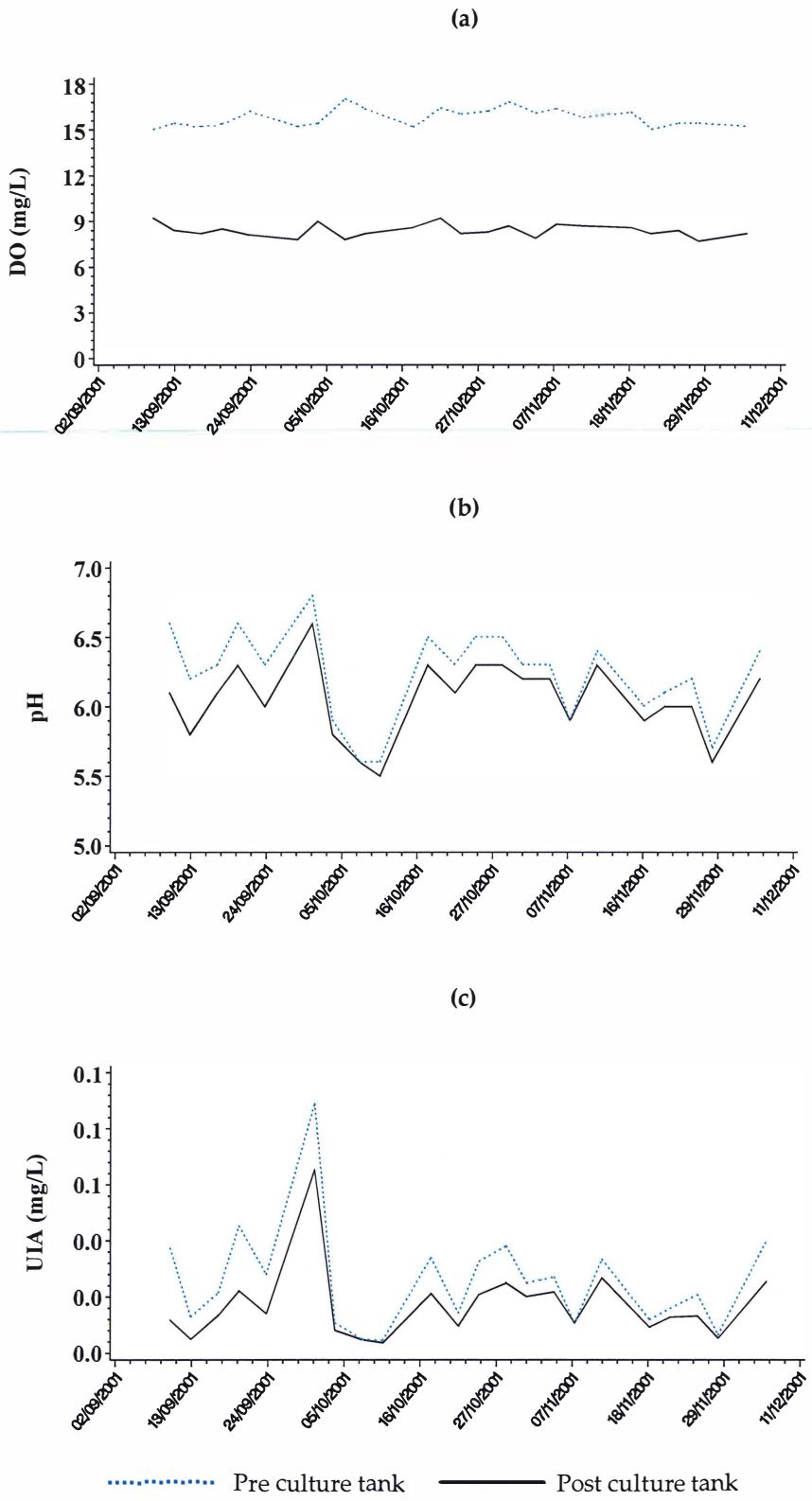

Fig. 5.2. Water quality in pre culture tank and post culture tanks water samples. (a) Dissolved oxygen. (b) pH. (c) UIA.

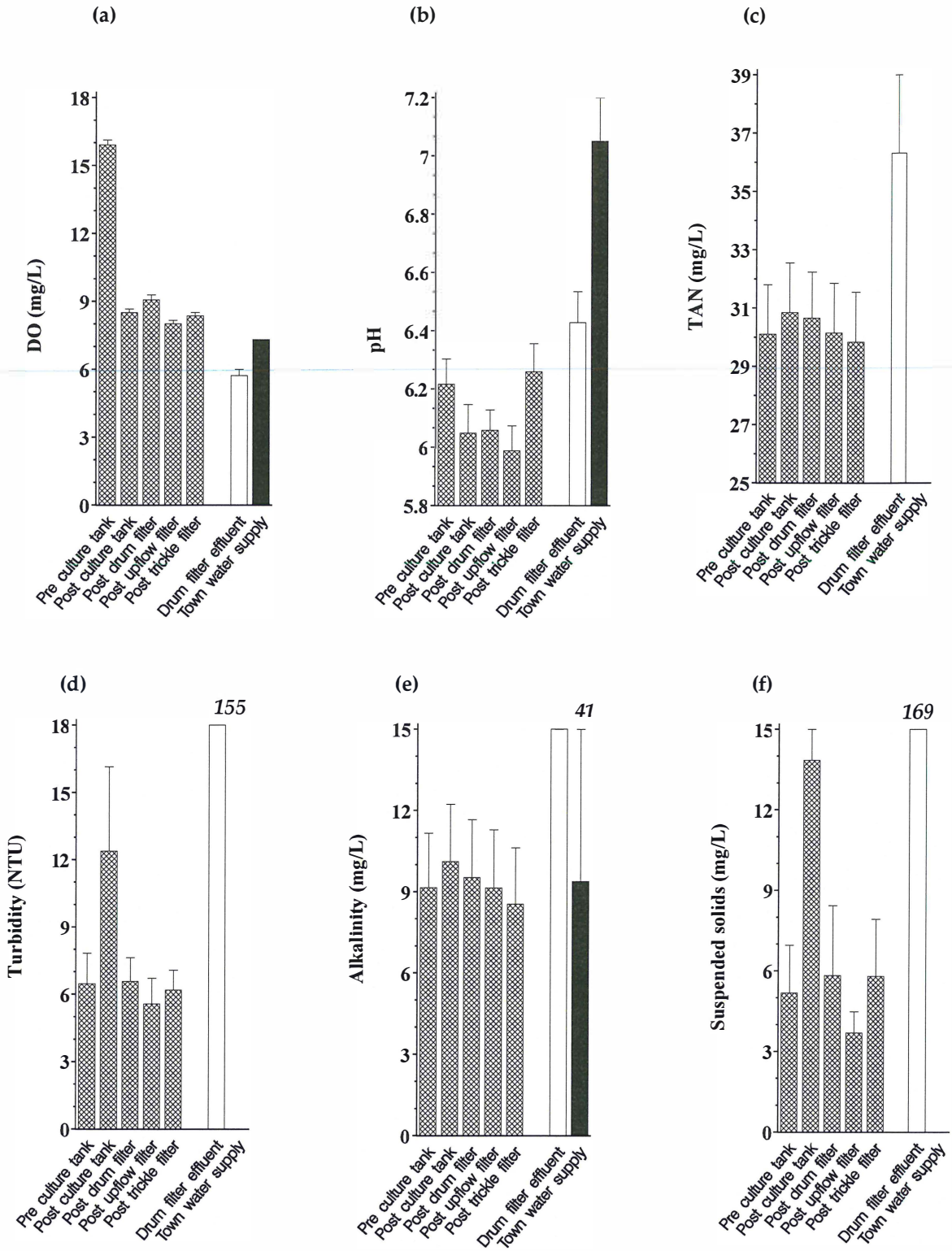

Fig. 5.3. Comparison of water quality values (mean + 95% confidence limits) measured from different sampling points within the RAS. (a) DO. (b) pH. (c) TAN. (d) Turbidity (drum filter effluent = 155 NTU). (e) Total alkalinity (drum filter effluent = 41 mg/L). (f) suspended solids (drum filter effluent = 169 mg/L).

5.3.1.8 Alkalinity

Total alkalinity ranged from 0-28 mg/L (mean 9.1 mg/L) within the system whereas concentrations in the drum filter effluent were considerably greater, 11-120 mg/L (mean 44 mg/L). Alkalinity also varied between sample points with lowest concentrations being recorded in the post upflow filter samples (Table 5.1). Alkalinity was significantly (P=0.02) affected by passage through both the drum filter and the upflow filter (Fig. 5.3e).

5.3.1.9 BOD

BOD within the treatment system showed variation between components and over time. Within the system, BOD readings were highest in the post culture tank samples (mean 17.5 mg/L) and lowest in the pre culture tank samples (mean 8.7 mg/L) (Table 5.1). BOD readings were significantly increased following passage through the culture tanks, and significantly reduced following passage through the upflow filter. BOD concentrations were considerably greater (mean 47 mg/L) in the drum filter effluent than the system.

5.3.1.10 Suspended and dissolved solids

Suspended solids, which ranged from 0.2-54 mg/L (mean 5.7 mg/L), were greatest in the post-culture tank water samples and lowest in the post upflow filter samples on all sampling occasions (Table 5.1). The highest suspended solids reading of 54 mg/L, which was recorded in the post culture water (13th October 2001) was thought to be associated with a fish grading event two days earlier. Suspended solids concentrations appeared to increase when fish were disturbed, such as by grading events. The drum filter significantly (P <0.0001) reduced suspended solids concentrations (Fig. 5.3f). The upflow filter also reduced suspended solids concentrations (Fig. 5.3f).

Dissolved solids ranged from 44 – 1,230 (mean 35 mg/L). Dissolved solids was affected by the addition of salt (NaCl), which was added to the system as a prophylactic during grading operations, and by increased water exchange during backwash events.

5.3.1.11 Carbon dioxide (CO_2)

CO_2 concentrations, which were determined on three occasions only, were greatest in post culture tanks samples (mean 29.7 mg/L) and post upflow filter samples (mean 30.4 mg/L) (Table 5.1). Lowest concentrations were found in post trickle filter samples (mean 25.4 mg/L), indicating the role of this filter in degassing of CO_2 from the system. Highest CO_2 concentrations were recorded in the drum filter effluent (mean 37.2 mg/L). CO_2 concentrations were negatively correlated with pH levels.

The upper limit of CO_2 concentrations recommended for fish in aquaculture is reported to be 10 mg/L (Table 5.3), yet concentrations 5 to 10 times this upper limit have been observed in RAS (Van Gorder 1995, in Vinci *et al.* 1996).

5.3.2 System components

5.3.2.1 Drum filter

The drum filter is the first component in the system to come into contact with water from the culture tanks (Fig. 5.1). It is an extremely important component that relies on a pump to spray debris off the drum-screen and a motor for rotation. The drum filter effectively removed about 58% of the suspended solids, thereby reducing turbidity by 48% and BOD by 23%.

Disposal of this water was initially via a drain into a paddock, however, the effluent pipe has since been connected to the domestic sewer system. It may be possible for this effluent stream to be treated with a separate up-flow filter to further reduce water loss from the system. Other options for disposal may be in some form of integrated agri-aquaculture system such as hydroponics or irrigation of pastures (Gooley and Gavine 2003). Indeed, such methods can be used to increase value of water, and feed being used in the system and can act as an additional source of income.

5.3.2.2 Upflow filter and trickle filter

The upflow filter is primarily used to remove finer suspended particles not removed by the drum filter, as indicated by the significant decline in suspended solids (Fig. 5.3). This process further evident when the filter is backwashed. The effluent discharged from the filter during weekly backwash operations is extremely high in total P (mean 43.4 mg/l), suspended solids (196 mg/l) and turbidity (1,340 NTU). The

decline in TAN, pH and DO following passage though the upflow filter indicates that some nitrification is occurring within the filter.

It has been speculated that some denitrification may also be occurring in anaerobic pockets within the upflow filter. However, the present study does not support this as NO_2-N and NO_3-N concentrations were not significantly changed by passage through the filter. Further, influent DO concentrations and the rate of flow of water though the filter may not allow for anaerobic pockets to develop within the filter.

TAN concentrations in post upflow filter and post trickle filter water samples were generally lower than in post-culture tank water samples (Fig. 5.4), suggesting that some nitrification was occurring within the filtration system. Both pH and temperature play a role in nitrification. Lekang and Kleppe (2000) showed that at temperatures of 5-15°C, highly efficient trickle filters remove up to 1.8 g total ammonia-N per m^2 per day. Lawson (1995) states that the optimum pH range for *Nitrosomonas* and *Nitrobacter* is 6-9 and 6.3–9.4, respectively, while Hagopian and Riley (1998) indicated that optimal temperature and pH for nitrification was 25°C and 7.8, respectively. Ammonia removal due to lower metabolism of the bacteria in the filter may be impeded by the lower temperatures recorded within the system during the present study (mean 19.8°C). In addition, the low pH readings observed in the present study (mean = 6.3 in post trickle filter) would lower the ability of bacteria to remove ammonia thereby further decreasing filter efficiency. Water turnover in the trickle filter is approximately once per hour, and the ammonia removal rate is 0.75 g total ammonia-N m^2 SSA/ day. This is approximately 40% of the value cited by Lekang and Kleppe (2000).

One of the major functions the trickle filter appears to have is the degassing of CO_2. Concentrations of CO_2 decline following passage through the filter, which in turn, is reflected in an increase in pH levels. Further, the acidifying effect of nitrification in the trickle filter has little impact on post trickle filter water samples due to over compensation by the degassing of CO_2. Degassing of the trickle filter may also be responsible for stripping N_2 (gas) (volatilisation) from the system.

The high amount of contact that the trickle filter has with air, due to high void space, is the reason that such effective degassing occurs, however, this can create a problem with water loss. Indeed, it has been suggested that up to 5,000 L/day of water may be lost by evaporation to the atmosphere via the trickle filter vents (P. van Lierop, *pers comm.*). Water being lost by this means is most evident on cool days when water vapour can be seen coming from the exhaust vents above the trickle filter. This loss in water also represents a loss of heat. One possible solution would be to use a condenser to reclaim water, while ensuring CO_2 degassing is not reduced.

5.3.3 Effluent discharge

A summary of water quality data for all parameters measured in the drum filter effluent during the present study are presented in Table 5.1. In general, nutrient concentrations, TAN, NO_2-N, NO_3-N and total P were substantially higher in the drum filter effluent than the system. DO was lower in the drum filter effluent than the system whereas BOD, suspended solids, turbidity and alkalinity were considerably higher.

5.3.3.1 Simplified nutrient mass balance model (Model A)

Inputs and outputs of Model A are summarised in Fig. 5.5. All output values for model one are presented as kilograms of nutrients (P or N) per tonne of Murray cod produced (ie kg /tonne). P and N in the effluent waters from fish farms is primarily derived from feed waste (dust and excess feed not eaten by fish) and faecal waste from the fish. Based on a food conversion ratio (FCR) of 1.3 which is typical of intensive Murray cod grow-out (Ingram 2004), approximately 1,300 kg of feed is required to produce 1 tonne of Murray cod. "Nova ME" (45% protein, 20% lipid), a high-energy barramundi feed produced by Skretting, Tasmania, is commonly used for the grow-out of Murray cod. This diet contains 1.0 – 1.6% (median 1.3%) total P and approximately 7.2% N. Based on these percentages, the amount of P and N in the feed required to produce 1 tonne of Murray cod is 16.9 kg and 94 kg respectively. Between 2% and 15% of the feed added to a trout farming system is not consumed by fish (GBWQWG 1995). Based on a median value of 6.5%, 15.8 kg P/t and 88 kg N/t was consumed by Murray cod while 1.1 kg P/tonne and 6 kg N/tonne was not consumed.

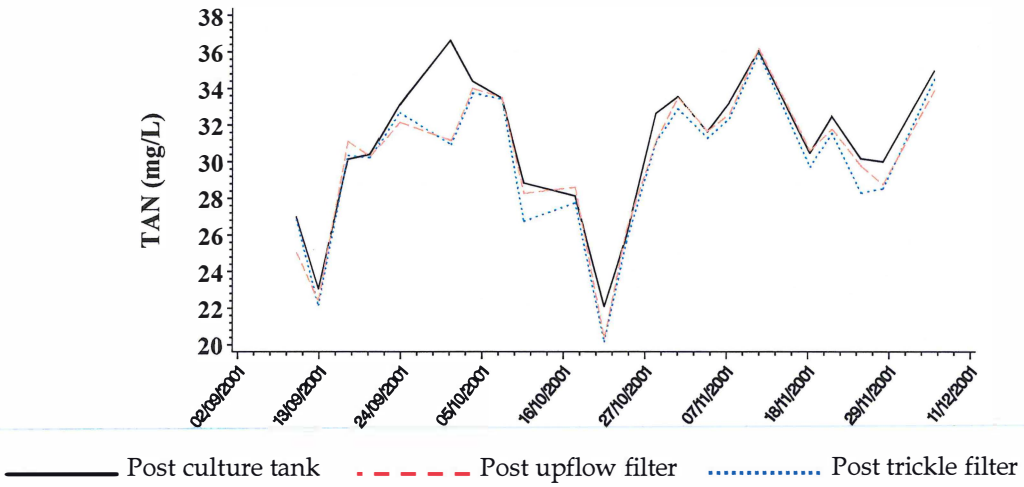

Fig. 5.4. TAN concentrations in post culture tank, post upflow filter and post trickle filter water samples recorded during the present study.

The P and N content of whole fresh fish is approximately 0.4-0.5% (median 0.45%) and 2.3 –3.0% (median 7%) of fresh weight (Lall 1991; Wallin and Håkanson 1991; Ramseyer 2002). Therefore, 4-5 kg P (median 4.5 kg) and 23-30 kg N (median 27 kg) is removed from the system for every tonne of Murray cod that is harvested. The balance of P and N is bound up in faecal and excreted wastes, being 11.3 kg P/tonne and 61 kg N/tonne (Fig. 5.5). Nutrients discharged from the system equals the sum of nutrients in uneaten feed, and faecal and excreted wastes. Based on these figures, for every tonne of Murray cod produced, approximately 12.4 kg P/tonne and 67 kg N/tonne are discharged as waste (Fig. 5.5). These values are strongly influenced by FCR and the amount of N and P in the feed. At a FCR of 1.1, 9.8 P/tonne and 52 kg N/tonne are discharged, whereas at a FCR of 1.5, 15 kg P/tonne and 81 kg N/tonne are discharged (Table 5.2).

Table 5.2. Effects of P and N content of feed and FCR on the amount of P and N discharged per tonne of fish produced

	Phosphorus (P)				Nitrogen (N)		
P in Feed	*FCR*			*N in Feed*	*FCR*		
(%)	*1.1*	*1.3*	*1.5*	*(%)*	*1.1*	*1.3*	*1.5*
1.0	6.5	8.5	10.5	*6.9*	49	63	77
1.3	9.8	12.4	15.0	*7.2*	52	67	81
1.6	13.1	16.3	19.5	*7.5*	56	71	86

5.3.3.2 Simplified water budget (Model B)

Estimates of the amount of P discharged were determined using estimates of P in the effluent water (drum filter effluent) measured during the present study and the volume of water discharged from the RAS annually (Fig. 5.6). The RAS in the present study, had a water capacity of approximately 172 M^3, about 20% of which was replaced daily with water from the town water supply. The majority of water discharged from the system was via the drum filter effluent line. However, a quantity of water is also lost via evaporation from the trickle filter (Peter van Lierop *pers comm*). Concentrations of total P in the drum filter effluent was 7.1-42.5 mg/L (median 19 mg/L) (Table 5.1). Based on these assumptions, approximately 12,556 M^3 water is discharged per year. At an annual production level of 25 tonne fish, this system discharges 3.6 – 21.3 kg P/tonne fish (Median 9.5 kg P/tonne fish) (Fig. 5.6).

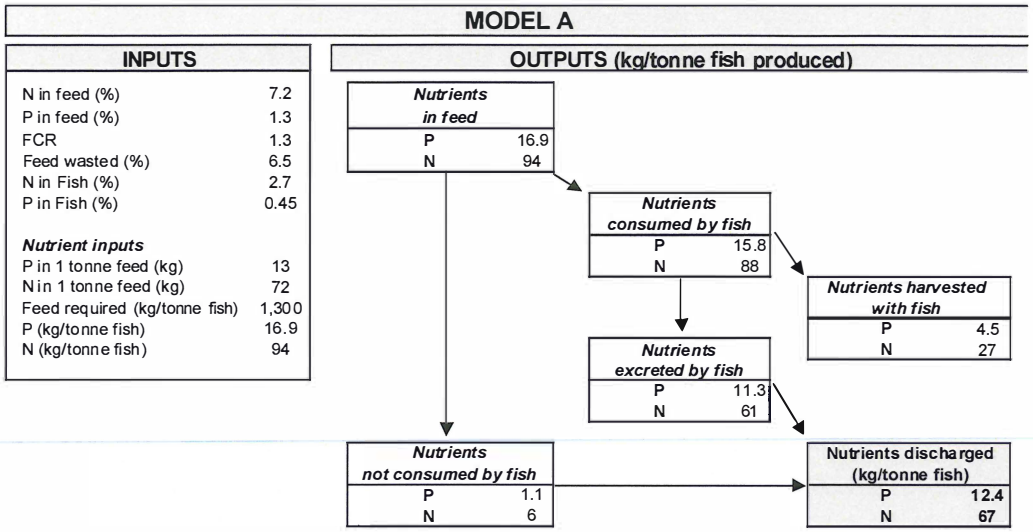

Fig. 5.5. Simplified nutrient mass balance model for estimation of phosphorus and nitrogen discharged from a RAS farming Murray cod

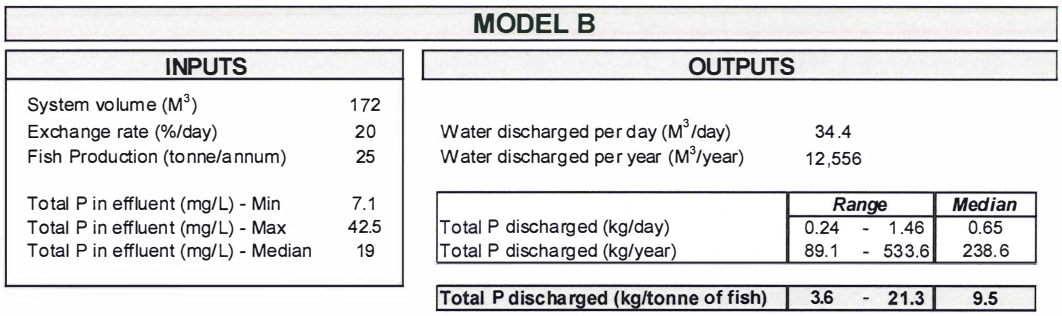

Fig. 5.6. Simplified water budget model for estimation of phosphorus discharged from a RAS farming Murray cod.

There was a sizeable difference in the estimates of P discharged from the two models. The median amount of P resulting from the production of one tonne of Murray cod in Model B (9.5 kg/tonne) was considerable less than that in Model A (12.4 kg/tonne). This may be due, in part, to the fact that much of the uneaten feed is removed from screens within the culture tank by hand daily, and so does not contribute to the effluent stream of the drum filter.

The amount of gaseous nitrogen (N_2) that is being discharged to the environment is unknown, but may be negligible as there is little denitrification occurring within the systems.

For every tonne of rainbow trout produced by the established trout farming industry in the Goulburn-Broken River catchment, 12.9 kg P/tonne and 38 kg N/tonne (FCR 1.24, 1.4% P and 5.5% N in feed) is discharged (Ingram 1999a; 1999b). The P discharged by Murray cod aquaculture in RAS (Model A and B) compared favourably with these trout discharge estimates, but N discharged by Murray cod aquaculture in RAS was substantially greater, primarily due to the higher level of N in the feed.

5.3.4 Water quality requirements for commercial Murray cod aquaculture

There have been no reported attempts to determine the optimal water quality requirements for Murray cod, either in the wild or under aquaculture conditions. Therefore, in order to develop realistic water quality

guidelines for the intensive production of Murray cod in RAS, water quality data collected during the present study and data collected since 1999 as part of the project, *Development of Intensive Commercial Aquaculture Production Technology for Murray Cod* (FRDC Project No. 1999/328) (see Ingram 2004) were combined to establish a water quality database for Murray cod aquaculture. To eliminate the effects on extreme values, data were summarised and presented here as median and 25%-75% interquartile range (Table 5.3). These values provide a guide only to what may be considered acceptable ranges for commercial intensive Murray cod aquaculture, however, these values must be treated with caution as there may be circumstances or conditions in which ranges for some values may be inappropriate for Murray cod. As a comparison, an amalgamation of values presented for other species (Shepherd and Bromage 1988; Boyd 1990; Rowland 1995; Piper *et al.* 1998) and water quality guidelines for the production of aquatic organisms for human consumption (ANZECC 2000) are also presented (Table 5.3). Most parameters presented for Murray cod aquaculture fell within the ranges suitable for "continuous exposure" (various sources). However, the interquartile range of TAN, total alkalinity, CO_2, total hardness, nitrite and BOD, median values for total alkalinity, CO_2 and nitrite, fell outside the ranges suitable for "continuous exposure" (various sources) (Table 5.3).

Table 5.3. Acceptable water quality ranges for commercial intensive Murray cod aquaculture

Parameter	Suitable concentrations (continuous exposure) (Various sources[1])	Recorded from Murray cod aquaculture[2]	
		25%-75%	Median
Water temperature (°C)	5-30	20-25	24
Dissolved oxygen (mg/L)	>5	6.9-8.8	7.8
pH	5.5-9	5.8-6.5	6.1
Total ammonia (as nitrogen) (TAN) (mg/L)	<3.0	0.3-26.5	2.9
Unionised ammonia (UIA) (mg/L)	<0.02 (pH>8.0) <0.01 (pH<8.0)	0.003-0.02	0.008
Total alkalinity (mg/L)	20-400	8-39	15
Carbon dioxide (mg/L)	<10	20-39	31
Chlorine (mg/L)	<0.003	No data	
Conductivity (us/cm)	<10,000	670-960	836
Total hardness (mg/L)	20-400	205-440	273
Hydrogen sulphide (mg/L)	<0.002	No data	
Nitrate (mg/L)	<100	27-77	44
Nitrite (mg/L)	<0.1	0.01-0.63	0.24
Phosphorus (mg/L)	No data	0.3-13.0	5.1
Suspended solids (mg/L)	<25	3.1-8.0	4.8
Dissolved solids (mg/L)	No data	171-424	244
Turbidity (FAU)	<25	3-10	5
BOD (5 day) (mg/L)	<15	2-23	13
COD (mg/L)	<15	No data	
Metals Aluminium (mg/L)	<0.03 (pH>6.5) <0.01 (pH <6.5)	No data	
Cadmium (mg/L)	<0.003	No data	
Calcium (mg/L)	10-160	No data	
Copper (mg/L)	<0.005	No data	
Iron (mg/L)	<0.05	No data	
Lead (mg/L)	<0.01	No data	
Magnesium (mg/L)	<15	No data	
Manganese (mg/L)	<0.01	No data	
Mercury (mg/L)	<0.001	No data	
Zinc (mg/L)	<0.01	No data	

1. Shepherd and Bromage (1988), Boyd (1990), Rowland (1995), Piper *et al.* (1998) and ANZECC (2000).
2. Summary of data collected during the present study, and the project entitled *Development of Intensive Commercial Aquaculture Production Technology for Murray Cod* (FRDC Project No. 1999/328), data presented as 25%-75% interquartile range and median.

5.4 Conclusion and recommendations.

The results from the present study showed that water quality within RAS is complex and dynamic, and that understanding how specific parameters are affected by the components (filters and culture tanks) within the system, other water quality parameters and farming operations, is critical to the overall management and performance of RAS. There is a high degree of inter-relationship between water quality parameters, which means that a variation in one parameter can influence the toxicity of others. The results of the present study have implications for the future construction, operation and management of RAS systems for the culture of Murray cod.

The water treatment components within the RAS, although usually performing the tasks they were designed for, also had strong influences on other water quality parameters. Nitrification occurred in both the trickle and upflow filters. The upflow filter was also efficient in trapping fine suspended solids, thereby lowering BOD and turbidity.

The trickle filter removed approximately 3.5 kg of ammonia per day. This filter also significantly influenced pH and UIA levels within the system. Generally trickle filters reduce pH, (as a result of nitrification) however in this study the reverse occurred. This was thought to be due to the degassing of CO_2 in the trickle filter which altered the carbonate equilibrium causing a rise in pH, and an associated elevation of UIA. Degassing is an important process being undertaken by the trickle filter, and is vital to the overall operation of the system. Systems must be designed to balance CO_2 stripping and pH control to maximise nitrification. This may require larger trickle filters that remove more ammonia or shifting the pH range toward 7 to allow improved nitrification.

The drum filter was an efficient and important water treatment component within the system as it significantly improved water quality by reducing suspended solids, BOD and turbidity. The drum filter was also the primary effluent discharge point for the system. The effluent from the RAS contained high concentrations of nutrients and suspended solids. Use of a nutrient mass balance model and a water budget model have provided estimations of the amounts of N and P derived from production of Murray cod in RAS. Although, due to the nature of RAS, small volumes of water are discharged relative to the level of production, cost effective and environmentally sound options for treatment and disposal of this waste stream need to be explored. These may include improvements to the water treatment process especially the incorporation of phosphorus stripping and denitrification technology, and utilising the discharged water for irrigation purposes.

Alkalinity is consumed when ammonium is converted to nitrate during nitrification (Bisogni and Timmons 1994). Low alkalinity within the system may have inhibited nitrification within the trickle filter and also influenced both pH and CO_2 concentrations. Improved nitrification in the system may be achieved by regular dosing of the pre trickle filter water with sodium bicarbonate or other buffering compounds. Further investigation into the effects of alkalinity on RAS is required.

This study provided valuable baseline information on water quality conditions in which Murray cod are being cultured, and presented ranges for some parameters that may be used as a guideline for the intensive production of Murray cod in RAS. However, these results do not provide an indication as to whether or not concentrations of the parameters measured affected growth of fish. For example, median values for total alkalinity, CO_2 and nitrite, fell outside the ranges that would be considered suitable for "continuous exposure" of aquatic animals. There is still a crucial need to identify critical ranges for optimal production of Murray cod under commercial conditions for some parameters. In particular, studies are required to determine the effects of various parameters that have the potential to influence growth and survival of fish in RAS, especially DO, CO_2, pH, UIA and nitrite.

5.5 Acknowledgments

The authors wish to thank first and foremost Peter and Carla van Lierop for allowing this study to proceed, for assisting during day-to-day sampling and for their generosity and hospitality. Staff at both PIRVic Snobs Creek and Deakin University, Warrnambool are thanked for their assistance during the project.

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6 DISEASES AND HEALTH MANAGEMENT IN INTENSIVE MURRAY COD AQUACULTURE

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CONTENTS

6.1 Introduction

Murray cod farming industry is expanding within Australia, particularly in eastern states. Although the production of fingerlings for stock enhancement purposes is well-established with fingerlings being commercially available since early 1980's, growout of Murray cod for human consumption under aquaculture conditions has only been undertaken since the mid 1990's (Ingram and Larkin 2000; Ingram *et al.* 2004).

The industry currently employs a range of fish holding and culture facilities and husbandry techniques from earthen ponds for holding broodfish and extensive growing of fry and fingerlings under ambient conditions to plastic and fibreglass tanks for intensive grow-out and purging under environment control conditions (Ingram and Larkin 2000).

Disease outbreaks have long been recognised as a significant constraint to aquaculture production and economic viability. Not only can disease outbreaks inflict heavy mortalities of stock, but fish in poor health have considerably lower growths rates which increases the time and costs to grow them to a marketable size. Some diseases can disfigure or render the fish unsightly which reduces their marketability. With intensification of culture conditions and increasing densities of fish, the spread of diseases, when they occur, is often more rapid and devastating in terms of mortalities.

There are numerous articles and general text books that deal with fish diseases in aquaculture, and there have been several key fish health workshops and associated publications undertaken within Australia that provide information on the identification, treatment and management of diseases (Humphrey and Langdon 1985; Bryden 1988; Munday 1990; 1992). At a national level, a strategic plan for aquatic animal health

('AQUAPLAN') and an aquatic animal disease veterinary emergency plan ('AQUAVETPLAN') have been developed (Anon 1999; Bernoth *et al.* 1999). However, fish health information for Murray cod is limited. Rowland and Ingram (1991) described the parasitic and fungal diseases of Murray cod, golden perch and silver perch, and a brief overview of diseases of Murray cod was given by Missen and Dobson (2000). There is a need to identify fish health issues in intensive Murray cod aquaculture and provide management strategies to assist in reducing the incidence and severity of disease outbreaks and health problems thereby ensuring continued long-term survival and growth of fish in aquaculture.

The aims of the present report were to:

(a) Identify key issues that affect the health of intensively farmed Murray cod.

(b) Provide information and strategies to assist farmers in the management of the health and well-being of stock in intensive aquaculture facilities.

6.2 Fish health survey of Murray cod industry

During June 2001, a survey was conducted of the Murray cod Aquaculture Network (MCANet) (see Ingram and Lawson 2004), to identify the major issues affecting the health of Murray cod in aquaculture. A total of 82 questionnaires were returned, 46 of which were practicing fish farmers with Murray cod on-site. All respondents had faced some fish health problems. Results from the survey (Fig. 6.1) indicated that the most commonly encountered health problems in Murray cod aquaculture were:

- ***Protozoan parasite infestations*** (28% of respondents), particularly by the ciliated ectoparasites *Chilodonella*, *Trichodina* and *Ichthyophthirius multifiliis* (white-spot), and the flagellated protozoan *Ichthyobodo*. Other protozoan parasites were infrequently encountered.
- ***Water quality*** (28% of respondents), including problems with dissolved oxygen, gas supersaturation, build-up of metabolic wastes (ammonia), and toxic contaminants.
- ***Fungal infections*** (19% of respondents), predominantly saprolegniasis.
- ***Non-specific bacterial infections*** (17% of respondents). In most cases, specific bacterium involved were not isolated or identified.
- ***Nutritional problems*** (11% of respondents), including swollen yolk sac syndrome, fatty liver syndrome, feed quality, feed composition and feed management.
- ***Predation/cannibalism*** (11% of respondents). Predation by water birds in open ponds (juveniles only) and cannibalism in ponds and tanks.

6.3 Diseases and parasites of Murray cod

6.3.1 Viruses

Viruses can induce diseases resulting in important economic losses in aquaculture. In Australia, several fish viruses have been reported including epizootic haematopoietic necrosis virus (EHNV), an iridovirus, the picorna-like barramundi virus and a birnavirus from Atlantic salmon. To date, there has been one confirmed case of a virus infecting Murray cod. In February 2003, an iridovirus-like virus was observed from the spleen and kidneys and gills of juvenile Murray cod held in a recirculating aquaculture system (Lancaster *et al.* 2003). This infection resulted in 90% mortality in fingerlings (40-60 mm) and 25% mortality in larger fish (100-150 mm).

Juvenile Murray cod have also been experimentally infected with EHNV, which has caused mass mortalities of juvenile redfin perch in the wild (Langdon 1989). Under laboratory conditions infected Murray cod were found to be less susceptible to EHNV than other experimentally infected freshwater fish species, but were nevertheless potential carriers of the virus. EHNV is a potentially serious problem in aquaculture and as such is a notifiable disease both nationally and internationally. Consequently, this virus should be considered as a potential threat to the farming of Murray cod and precautions should be taken to prevent its introduction in aquaculture facilities.

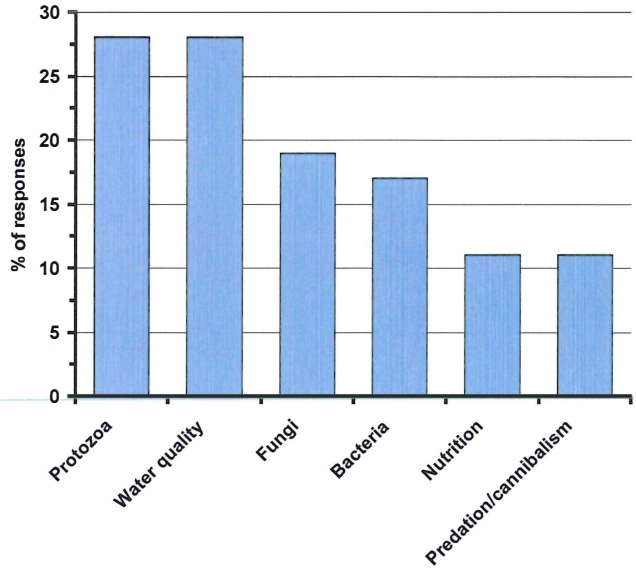

Fig. 6.1. Fish health problems encountered in Murray cod aquaculture (percent of respondents from industry survey).

6.3.2 Bacteria

Numerous bacterial organisms have been recorded from fish (Roberts 1982; Munday 1989; Carson 1990; 1992). Often, however, many of these occur as secondary infections and involve bacterial organisms which are normal inhabitants of the aquatic environment, but only cause disease following stress-induced factors such as parasitic infestation, changing environmental conditions (temperature, pollution etc.) and poor husbandry techniques.

Survey of the MCANet indicated that bacterial infections have occurred in Murray cod aquaculture operations. However, there are few published reports describing the specific pathogens associated with these infections (see Table 6.1). However, farmers have reported incidences of 'fin rot'- or 'tail rot'- and 'saddleback'-like diseases. These diseases are typically caused by *Cytophaga*- and *Flexibacter*-like bacteria ("myxobacteria"), in particular *Flexibacter columnaris*, which infects the gills, body surface and fins of fish, especially juveniles. In severe infections by these bacteria the skin and/or fins become severely eroded and acute infections may lead to mortality.

Aeromanas salmonicida is responsible for furunculosis, a serious and important disease of farmed salmonids overseas. In Australia, Goldfish Ulcer Disease (GUD), which is caused by the atypical strain *Aeromanas salmonicida nova*, which was first recorded in Australia in 1974, and has been reported from carp goldfish and silver perch (Whittington *et al.* 1987; Callinan and Rowland 1995). Fish infected with GUD develop haemorrhagic lesions on the skin (Plate 4a). Murray cod are apparently resistant to GUD, but may act as carriers without showing clinical signs of it's presence (R. Whittington *pers comm*, in Rowland and Ingram 1991).

Biofilms form on all surfaces within aquaculture facilities. Indeed, bacterial biofilms in biological filters are responsible for nitrification processes. Biofilms may also contain pathogenic micro-organisms, which may also pose a health to humans through direct exposure and via processed fish products. Pathogenic bacteria, including *Aeromonads*, *Vibrios*, *Yersinias* and *Bacillus cereus*, have been isolated from recirculating aquaculture systems, but whether the presence of these bacteria pose a health risk is uncertain (King and Flick 2000).

Table 6.1. Parasites and diseases recorded from farmed and wild Murray cod.

PARASITE/DISEASE		SOURCE	FISH STAGE & SITE	REFERENCES
VIRAL	Epizootic haematopoietic necrosis virus (EHNV)	Laboratory only	Internal organs	Langdon (1989)
	Hypertrophy iridovirus	Farmed	Internal organs	Lancaster *et al.* (2003)
BACTERIA	*Myxobacteria*	Wild	No data available	L. Ashburner *pers com* in Rowland and Ingram (1991)
	Aeromonas salmonocida nova (GUD)	Laboratory only	Body surface	R. Whittington *pers com* in Rowland and Ingram (1991)
FUNGI	*Saprolegnia*, & *Achlya* (Oomycete fungi)	Farmed	All life stages. Body surface & gills	Rowland and Ingram (1991)
PROTOZOA	*Chilodonella piscicola* (= *C. cyprini*)	Farmed	Larvae, juveniles & adults. Gills	Ashburner and Ehl (1973)
	Chilodonella hexasticha	Farmed	Larvae, juveniles & adults. Body surface & gills	Rowland and Ingram (1991)
	Epistylis	Farmed	Sub-adults. Body surface	PIRVic, Snobs Creek; Halliday and Collins (2002)
	Goussia lomi	Farmed	Juveniles. Mucosa of intestine	Molnar and Rohde (1988) Philbey and Ingram (1991)
	Ichthyobodo necator (= *Costia necatrix*)	Farmed	Larvae, juveniles & adults. Body surface & gills	Rowland and Ingram (1991)
	Ichthyophthirius multifiliis (white spot)	Farmed	Larvae, juveniles & adults. Body surface & gills	Rowland and Ingram (1991)
	Myxosoma	Farmed	Juveniles & adults. Gills	Ashburner (1978)
	Tetrahymena	Farmed	Juveniles. Body surface & gills	Rowland and Ingram (1991)
	Trichodina	Wild & Farmed	Larvae, juveniles & adults. Body surface & gills	Rowland and Ingram (1991)
METAZOA	*Clinostomum complanatum*	Farmed	Juveniles. Body cavity & eye	Anon (2001)
	Diplostomum Spathaceum	Wild	Juveniles & adults. Lens of eye	Johnston and Angel (1941)
	Stegodexamene watsoni	Wild	Intestine	Cribb (1988)
	Capillaria murrayensis	Wild	Intestine	Johnston and Mawson (1940)
	Contracaecum sp.	Wild	Mesentry & omentum	Johnston and Mawson (1940); Johnston and Mawson (1947)
	Goezia fluviatilis	Wild	Gill mucus & intestine	Johnston and Mawson (1940); Johnston and Mawson (1951)
	Hysterothylacium murrayense	Wild	No data available	Johnston and Mawson (1940)
	Spinitectus sp.	Wild	No data available	Johnston and Mawson (1940)
	Ergasilus intermedius	Wild	Gills	Kabata (1992) (see also Ingram and Philbey 1999)
	Lernaea sp.	Wild & farmed	Juveniles & adults. Body surface	Ashburner (1978); Rowland and Ingram (1991)
	Histiostoma papillata	Farmed	Juveniles. Body surface & gills	Halliday and Collins (2002)
	Hydrozetes sp.	Farmed	Juveniles. Body surface	D. Walters, *pers comm* in Halliday and Collins (2002)
OTHER	Fatty liver syndrome	Farmed	Juveniles. Liver	PIRVic, Snobs Creek
	Chronic erosive dermatopathy (CED)	Farmed	Juveniles & adults. Body surface	Humphrey *et al.* (2000); Trott (2000); Baily (2003)
	Blue-sac syndrome	Farmed	Eggs and larvae	Gunasekera *et al.* (1998)
	Hypoxia	Wild		McKinnon and Shepheard (1995)
	Gas bubble disease	Farmed	Larvae, juveniles & adults	PIRVic, SnobsCreek

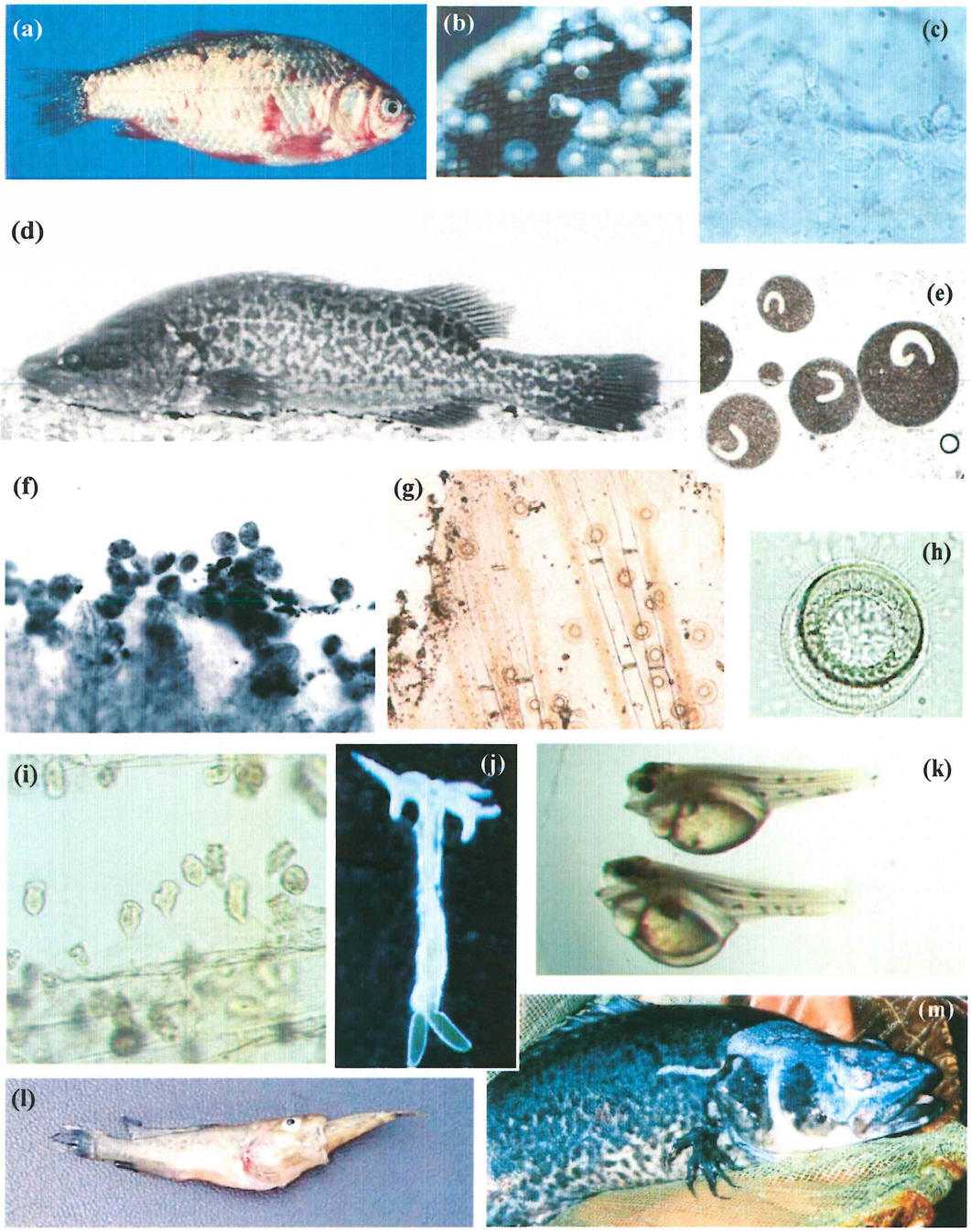

Plate 4. Common diseases and parasites of farmed Murray cod. (a) goldfish infected with *Aeromanas salmonicida nova.* (b) fungus growing on Murray cod eggs (c). *Ichthyobodo necator.* (d) Murray cod infested with *Ichthyophthirius multifiliis.* (e) *Ichthyophthirius multifiliis* trophonts. (f) *Chilodonella* on gill filaments. (g) *Trichodina* on fin of juvenile Murray cod. (h) *Trichodina.* (i) *Epistylus* on fin of juvenile Murray cod. (j) *Lernaea.* (k) swollen yolk sac syndrome in Murray cod larvae. (l) Cannibalism in juvenile Murray cod. (m). Chronic erosive dermatopathy in Murray cod.

6.3.3 Fungi

Murray cod farmers often encounter fungal infections in their stock (Fig. 6.1). Most fungal infections recorded from fish are caused by species belonging to the oomycete fungi, in particular *Saprolegnia, Achlya* and *Aphanomyces* (Neish and Hughes 1980). Diseases caused by these fungi are collectively called "saprolegniasis". Oomycete fungi, which are ubiquitous and common in moist environments, are rarely considered to be primary disease pathogens, but are more often classed as saprophytic opportunists that will readily colonise damaged tissues infected by bacteria or parasites, and dead or decaying matter. Fungal growths appear as patches of white to whitish-grey cottonwool like growths, which are composed of numerous hyphae. Murray cod eggs are particularly prone to attack by fungi (Plate 4b). The gills body surface and fins of larval, juvenile, sub-adult and adult Murray cod are also prone to fungal infestations (Rowland and Ingram 1991).

Epizootic ulcerative syndrome (EUS) has not been reported from Murray cod. However, since EUS is a severe and economically important disease affecting farmed freshwater fish it is listed as a notifiable disease in Australia, and significant outbreaks have already occurred in farmed silver perch in NSW (Callinan *et al.* 1999). EUS is characterised by the presence of the fungus *Aphanomyces* that invades the body surface (Lilley *et al.* 1998).

6.3.4 Protozoan parasites

Infestations by protozoan parasites are the most common and problematic diseases currently faced by Murray cod farmers (Fig. 6.1). In particular the ciliated ectoparasites *Chilodonella*, *Trichodina* and *Ichthyophthirius multifiliis* (white-spot), and the flagellated protozoan *Ichthyobodo*, are frequently recorded on larvae, fingerlings and adults.

6.3.4.1 Ichthyobodo

Ichthyobodo necator (formerly *Costia necatrix*) (Plate 4c) is a small flagellated protozoan (10-15 μm in length) which is an obligate parasite of fish. In crowded conditions *Ichthyobodo* spreads rapidly. Each cell bears two or four flagella which are used for location while not attached to the host. *Ichthyobodo* parasitises many species of fish and death from infestation is more likely in larvae and juvenile Murray cod than adults. *Ichthyobodo* infest the gills and body surface, including fins of larvae, juveniles, sub-adults and adults of a wide variety of freshwater fish species including Murray cod.

6.3.4.2 Ichthyophthirius multifiliis

Ichthyophthirius multifiliis ('ich' or 'white spot') is a large ciliated protozoan that has a complex lifecycle, which includes both free-living and parasitic stages. The trophont stage is parasitic and burrows under the upper layer of the gills, fins and skin (epithelial tissue). Trophonts are large cells (0.05-1.0 mm dia) that are clearly visible to the naked eye as white spots on the skin of infested fish (Plate 4d). These trophonts constantly rotate slowly underneath the skin, and sometimes the distinctive horseshoe-shaped macronucleus is visible (Plate 4e). Because the trophonts are beneath the surface of the skin, many therapeutics are ineffective. Instead, control of *Ichthyophthirius* relies on destroying the free-living stages and preventing re-infection. Outbreaks of this parasite have caused mass mortalities in a wide variety of freshwater fish on fish farms in Australia and overseas, and larvae and fry may die before trophonts are detected. All life stages (except eggs) of Murray cod are particularly susceptible to infection by this parasite and mass mortalities have been recorded in aquaculture facilities (Rowland and Ingram 1991).

6.3.4.3 Chilodonella

Chilodonella is a ciliated protozoan (50-70 μm in length), which includes free-living species and facultative and obligate parasite species (Plate 4f). Species of *Chilodonella* are distinguished from other ciliated parasites by the typical gliding movement, the flattened and slightly distorted oval shape body, the bottom surface is flat while the upper surface slightly domed or vaulted. The upper surface has a series of ciliary rows. Outbreaks of this parasite have caused significant mass mortalities of a wide variety of farmed freshwater fish worldwide (Sarig 1971). Murray cod are very susceptible to infestation by this parasite and mass mortalities of larvae, juveniles and broodfish held in both tanks and ponds have been recorded, and has been implicated in mortalities of Murray cod in the wild (Rowland and Ingram 1991).

6.3.4.4 Trichodina

Trichodina, a ciliated protozoan (25-100 μm in dia.) that infests the gills, body surface and fins of a wide variety of freshwater fish, is distinguished from other parasites by its typical circular shape and spinning swimming action (Plate 4g). The body is disc or bowler-hat-shaped and posterior possesses an adhesive disc containing denticles (hook-like structures) and is bordered with a ciliary band (Plate 4h). *Trichodina* is arguably the most commonly encountered parasite found on native fish in aquaculture facilities. Although *Trichodina* may not reach epizootic levels and be as virulent as *Chilodonella* and *Ichthyophthirius* infestations, mortalities in Murray cod are more likely to occur in larvae, fry and fingerlings that are heavily infested. Adults may carry the parasite without apparent affect. Infestations are often associated with poor water quality and fish in both ponds and tanks are affected.

6.3.4.5 Other protozoans

Other protozoan parasites were infrequently encountered on Murray cod in aquaculture facilities (Table 6.1). Sessile peritrichs (eg *Epistylis* [sic *Heteropolaria.*]), are ciliated protozoans that are mostly commensal in habit, attaching to hard surface by means of a holdfast or stalk (Plate 4i). Infestations of fish by sessile peritrichs are uncommon, but when they occur it is usually associated with poor water quality and high organic loading. Infestations by sessile peritrichs have been reported from the body surface and fins of Murray cod (Table 6.1). At PIRVic, Snobs Creek mortalities occurred as a result of *Epistylis* infestation in juvenile Murray cod held in a RAS. There have been unconfirmed, anecdotal reports of outbreaks of *Oodinium* causing mortalities in intensively farmed Murray cod.

6.3.5 Metazoan parasites

6.3.5.1 Trematodes and nematodes

Monogenean trematodes (flukes) mostly parasitise the body surface, and gills of fish. Dactylogyrids and gyrodactylids are the two most common monogeneans that are important parasites in aquaculture. However, infestations by these parasites have not been confirmed for Murray cod. In contrast, digenean trematodes are mostly internal parasites that have a complex lifecycle which may include several hosts including both aquatic invertebrates and vertebrates. Mature stages possess one anterior and one ventral sucker. *Clinostomum complanatum* (yellow grub) has been recorded from the body cavity and eyes of farmed juvenile Murray cod in Queensland (Anon 2001, W. Townsend, *pers comm*). These parasites, which are about 4-5 mm long, are visible to the naked eye as white coloured spots amongst the connective tissue and organs in the body cavity. Although this report did not indicate that significant mortalities occurred as a result of this the infestation, parasitism of farmed fish by *C. complanatum* is a concern as the presence of the highly visible grub is unsightly and may cause rejection of fish at the market. Further, there are numerous reports of humans being infected by *Clinostomum* following ingestion of uncooked freshwater fish harbouring the parasite (Isobe *et al.* 1994; Coulibaly *et al.* 1995).

Both the larval and adult stages of nematodes parasitise fish, and numerous species have been reported from native species, including several species from wild Murray cod (Table 6.1). However, nematodes are not often reported as being a serious problem in farmed fish.

6.3.5.2 Arthropods

Anchor worms (*Lernaea* spp.) are large (up to 22 mm) crustacean parasites that infest the skin and gills of freshwater fish especially during the summer months (Plate 4j). Larval and male anchor worms are not parasitic whereas the female is parasitic. Anchor worm is an appropriate name for this parasite as the head and mouthparts of the female become modified to form an anchor shape following attachment to the host. A small, red pustule forms at the site of penetration. The body of the parasite, with large greenish pair of egg sacs on the posterior end, can be clearly seen protruding from the wound. *Lernaea* have not been reported from Murray cod reared in intensive recirculating aquaculture systems, but are often seen on Murray cod broodfish and fish grown in outdoor earthen ponds and cages (Rowland and Ingram 1991; Ingram 2004). Light infestations of *Lernaea* may not be life threatening unless penetration by the parasite is near a vital organ. Heavy infestations may lead to debilitation and infection by bacteria and fungi.

Water mites are generally not considered as fish parasites. However, under certain environmental conditions they may proliferate and subsequently colonise weak or stressed fish. One species, *Histiostoma papillata* has been reported to attack and apparently kill juvenile Murray cod held in a recirculating aquaculture system (Halliday and Collins 2002). Mites were found on the fins, body surface and gills of

infested fish, which were listless and had difficulty swimming upright. The fins were damaged and showed signs of haemorrhaging at the bases.

6.3.6 Nutritional diseases

In aquaculture facilities, the primary source of nutrients for growth and survival of Murray cod are derived from artificial feeds manufactured by commercial feed companies. Nutritional requirements of fish, especially for protein, amino acids, lipids, minerals and vitamins, varies from one species to another, and for some species the nutritional requirements will also vary according to age. Nutritional deficiencies or imbalances can reduce growth rates and even cause disease in farmed fish. A specifically formulated, nutritionally balanced, artificial diet has been developed for the rearing of Murray cod in aquaculture facilities (see De Silva *et al.* 2004). However, to date this diet has not been taken up by commercial feed manufacturers.

Starvation may occur as a result of underfeeding (miscalculation of required feed rates or oxygen levels are too low to maintain optimal feeding rates) and/or use of inappropriate feed characteristics (diet composition, digestibility, palatability and size). Starvation may occur during the weaning of fingerling Murray cod. Fingerlings should be carefully selected for weaning as stressed fish or fish in poor condition are less likely to accept an artificial diet (Ingram *et al.* 2001)

Essential fatty acids (EFA) deficiencies can retard growth, cause nervous disorders and skin lesions and increased mortality. However excessive EFA in the diet can cause degeneration of the liver. Feeding high-energy lipid-rich diets to juvenile and sub-adult Murray cod in captivity over extended periods of time has caused loss of appetite, lethargy, deposition of excessive fat in the body organs, especially within the body cavity and liver ("fatty liver"), and ultimately death.

"Swollen yolk-sac syndrome" (SYSS) has caused significant mortalities of the eggs and newly hatched larvae of Murray cod in the hatchery at PIRVic. Larvae afflicted by SYSS are pale and possess a swollen and misshapen yolk sac as a result of fluid accumulation (Plate 4k), and usually die before exogenous feeding commences. Studies have shown that eggs affected by SYSS have significantly lower amounts of essential and non-essential amino acids than did normal eggs (Gunasekera *et al.* 1998), which suggests that SYSS is related to the nutrition of the broodfish held in earthen ponds.

6.3.7 Diseases associated with water quality

Survey of the MCANet indicated that fish health problems associated with water quality have occurred in Murray cod aquaculture operations (28% of respondents) (Fig. 6.1). Water is a vital component of any aquaculture venture as it is the medium in which the fish live and grow. Many water quality parameters, if they are altered beyond acceptable limits for the fish being cultured can influence fish health both directly and indirectly. Each species has a preferred range of water quality parameters and outside of this range will suffer stress, which may lead to disease and even death. There is a high degree of inter-relationship between water quality parameters, which means that a variation in one parameter can influence the toxicity of others.

Water quality requirements vary with species, but there is very little data on the tolerance levels of Murray cod or other native finfish. Shepherd and Bromage (1988), Piper *et al.* (1998), Boyd (1990) and Rowland (1995) provide useful summaries of water quality requirements for various species and forms of aquaculture. In the absence of specific water quality requirements for Murray cod, Boreham *et al.* (2004) (Table 5.3) have attempted to provide a guide to acceptable quality for the intensive production of Murray cod in RAS using historical water quality data from Murray cod aquaculture operations, which was compared against a summary of values published for other species and water quality guidelines for the production of aquatic organisms for human consumption (ANZECC 2000).

Water quality parameters that most commonly cause fish health problems in Australia include:

- Dissolved gases – particularly dissolved oxygen (DO). Other gases which may be important include: carbon dioxide; chlorine; hydrogen sulphide; methane and nitrogen.
- Metabolic waste products - particularly ammonia and nitrite.
- Other important parameters - pH, temperature, heavy metals, water hardness and salinity,
- Water-borne contaminants and other problems: toxic organic compounds, biocides, and noxious algae.

6.3.7.1 Dissolved gases

Environmental hypoxia is a low concentration of dissolved oxygen (DO) in the water and is perhaps the greatest risk in aquaculture. A minimum DO concentration of >5mg/L is considered ideal for optimal growth and reproduction. Below this level food consumption decreases and growth slows. However, DO concentrations of less than 2 mg/L have been recorded in fry rearing ponds used to rear Murray cod fingerlings (Ingram 2001). Problems with hypoxia in intensive Murray cod aquaculture facilities are being avoided by supersaturation of inlet water with pure oxygen (Boreham *et al.* 2004).

Gas supersaturation in intensive aquaculture operations occur in inflow water saturated with gases as a result of heating under pressure, water inflow pipes sucking in air (prior to pumps) and groundwaters that are supersaturated with nitrogen and/or carbon dioxide. Most gas emboli are produced by excess nitrogen. Oxygen rarely causes gas bubble disease because it is assimilated metabolically and thus less likely to form persistent bubbles. Gas bubble disease has occurred in juvenile and adult Murray cod held in RAS at PIRVic Snobs Creek. Infected fish were listless and floated to the surface, and small bubbles were visible the behind the eyes, gill filaments and fins.

The main sources of CO_2 in RAS are respiratory wastes of the fish and aerobic bacteria (primarily within the biological filter). High levels of free carbon dioxide (CO_2) have caused problems in RAS growing Murray cod. Boreham *et al.* (2004) reported CO_2 concentrations of between 20-39 mg/L in a RAS growing Murray cod, which were considerably higher than the recommended max concentration of 15 mg/L for continuous exposure (see Table 5.3 in Boreham *et al.* 2004). These results highlight the importance of degassing of CO_2 in RAS.

6.3.7.2 Metabolic waste products

Ammonia poisoning is one of the most common water quality problems in aquaculture. Ammonia is excreted by fish and if allowed to accumulate, can adversely affect water quality (Weirich *et al.* 1993). Ammonia is the primary nitrogenous waste product of fish, but also comes from the decay of organic matter such as waste feed. Ammonia in aqueous solutions occurs in two forms, unionised ammonia (UIA), which is toxic to fish, and ionised ammonia. The proportion of ammonia in the toxic unionised form is affected by water temperature and pH (Boyd 1990). Concentrations of UIA recorded from Murray cod aquaculture operations to date have generally been less than 0.02 mg/L (see TABLE 5.3 in Boreham *et al.* 2004) which is acceptable for continuous exposure.

Under aerobic conditions, ammonia is oxidised by bacteria to nitrite and then nitrate. Nitrate is generally considered non-toxic to fish, but nitrite is toxic under certain conditions (Lewis and Morris 1986; Weirich *et al.* 1993; Frances *et al.* 1998). Many of the circumstances that lead to a build up of ammonia can also lead to nitrite poisoning. Concentrations of nitrite less than 0.1 mg/L are considered suitable for continuous exposure, however, higher concentrations in excess of 0.6 mg/L have been recorded from Murray cod aquaculture operations (see TABLE 5.3 in Boreham *et al.* 2004).

Susceptibility to ammonia and nitrite toxicity varies enormously between species. There is no data on the toxicity of these chemicals to Murray cod, but studies are required to determine the effects of exposure to UIA and nitrite on growth of Murray cod in aquaculture facilities.

6.3.7.3 Other water quality parameters

Temperature dramatically affects fish metabolism and each species has a preferred temperature range. Absolute temperature ranges do not exist because tolerance depends on several factors, including the acclimation history, salinity, life stage and reproductive status. Under ambient conditions within the natural range Murray-Darling Basin, Murray cod may be exposed to temperatures from less than 10°C to in excess of 30°C. At the extremes, fish may be stressed to the point where growth and survival are affected. Not surprising, studies have shown that the growth of Murray cod is affected by water temperature. At temperatures below 16°C, feeding is reduced and growth is negligible (Ingram 2004). However, at higher temperatures, fish may stop feeding and become stressed, predisposing them to disease. Ingram (2004) attributed mortalities in Murray cod during a cage culture experiment, to stress and other factors associated with water temperatures that reached 30°C. The optimal water temperature for the culture of Murray cod is not clearly defined, however, under current industry practices Murray cod are being reared at temperatures between 20°C and 26°C in intensive RAS.

A pH range of 6.5-9.0 is generally recommended for freshwater fish. Fish acclimated to low or high pH levels, or fish used to pH fluctuations are more tolerant of pH changes than fish kept under more stable conditions. pH also affects the toxicity of other chemical parameters, such as ammonia (Boyd 1990). Murray cod being cultured in intensive aquaculture facilities are being exposed to pH's between 5.8 and 6.5 (see Table 5.3 in Boreham *et al.* 2004), whereas in fertilised earthen fry rearing ponds, juveniles are being exposed to pH's between 5.6 and 10.4 (Ingram 2001). The high pH readings reported in fry rearing ponds are the result of dense algal blooms removing CO_2 by photosynthesis (Boyd 1990). In contrast, in RAS there is a tendency for the pH to decrease as a result of respiration of fish and an associated build-up of CO_2 (Grace and Piedrahita 1994). However, operators of RAS often actively manage pH in RAS systems by stripping CO_2 and addition of a buffering agent such as sodium bicarbonate of sodium hydroxide (Bisogni and Timmons 1994; Grace and Piedrahita 1994). Managing pH is a balancing act. If pH is allowed to become too acidic, fish may become stressed and nitrification processes are impaired, whereas increasing pH will increase the proportion of ammonia that is toxic to fish.

6.3.8 Other fish health issues

6.3.8.1 Stress

Stress is considered as an environmental externality that reduces the ability or capacity of a fish to maintain health and well-being. More importantly, stress can reduce growth and illicit poor fish health (Iwama *et al.* 1997). Stressed fish suffer from depressed immune systems and consequently lowered resistance to disease or parasite infestation. Factors that may stress fish in aquaculture include handling, capture, netting, grading weighing, tagging, injecting, transport, territorial behaviour, crowding, chemicals, water quality and diseases. Some factors may not be stressful by themselves but combinations of several factors, acting in synergy, or even repeated and/or prolonged exposure to less than favourable conditions, may have an accumulative affect on fish health.

6.3.8.2 Aggression and cannibalism

Murray cod are known to be aggressive, territorial and predatory in nature, and it is not surprising that they are also capable of attacking and consuming their own kind. Cannibalism appears to be prevalent in culture tanks where densities are low and/or the size range of fish is great (Plate 4l). Other factors have also been shown to affect the rate and extent of cannibalism, including availability and nutritional composition of food, feeding frequency, water clarity, light intensity and the presence of refuges (Smith and Reay 1991; Hecht and Pienaar 1993). Increased turbidity may reduce territorial and aggressive behaviour and cannibalism. Murray cod are less likely to attack and consume siblings that are already dead. Cannibalism can lead to shifts in the size variation of stock and increase the risk of disease. Stress associated with cannibalism pressure may reduce growth rates in smaller fish and lead to increased susceptibility to disease. Larger fish may also suffocate while attempting to ingest smaller fish. The Murray cod aquaculture industry has generally overcome these problems by regular grading of fish, maintaining high densities and providing optimal feeding regimes, which appears to reduce the incidence of aggression and cannibalism and overall stress levels in cultured fish.

6.3.8.3 Predation

Many species of aquatic insects are predatory, such as the Aeshnidae (Odonata), Dytiscidae (Coleoptera) and Notonectidae (Hemiptera). In fry rearing ponds, these aquatic insects, particularly when abundant, can compete with fish for food such as zooplankton, or even prey directly on fish larvae and small fry. To date, however, there is limited information available on the potential impacts of predation by aquatic insects on the survival and growth of juvenile Murray cod in fry rearing ponds.

Birds that most frequently occur around aquaculture ponds and farm dams are the wading birds (herons and egrets), diving birds (cormorants and darters) and web-footed birds (ducks, grebes etc.). Some of these species are considered a pest in aquaculture ponds. The cormorants especially are notorious fish predators. Cormorants can eat up to 27% of their body weight per day, with daily intake ranging from 125g/day for little pied cormorants to in excess of 500g/day for black cormorants (Barlow 1995). Consequently, the impact of fishing by flocks of cormorants on fish in aquaculture ponds can be quite devastating

6.3.8.4 Eye disorders

Cultured fish occasionally develop eye disorders, the more common of these are exophthalmos (popeye), lenticulitis (cataracts) and keratitis (ketatopathy). Fish with exophthalmos typically have swollen or

protruding eyes and occasionally there may be gas bubbles present within the eye. Lenticulitis affects the lens of the eye, which becomes cloudy or opaque. In fish suffering keratitis the cornea of the eye is injured resulting in a dulling, cloudiness or discolouration of the cornea, inflammation and/or ulceration. The causes of eye disorders are varied and include nutritional deficiencies or imbalances, parasitism, poor water quality, gas supersaturation, presence of toxicants, physical trauma and stress associated with captivity and osmotic imbalances (Hargis 1991). Indeed the presence of some eye disorders may serve as an indicator of unfavourable culture conditions.

6.3.8.5 Chronic erosive dermatopathy

Chronic Erosive Dermatopathy (CED) was first identified as a problem on Murray cod in intensive aquaculture operations in the mid- to late- 1990's. CED is an ulcerative condition that affects the sensory pores and tissue overlaying the lateral line (Plate 4m). In extreme cases, the eyes and fins are also affected. Few mortalities appear to occur and fish continue to grow. The causative agent of CED has yet to be conclusively identified. Viruses, protozoan and metazoan parasites are not implicated. Bacterial pathogens are present but considered to be secondary/opportunistic colonisers of infected areas. Poor nutrition is not considered a key factor. Currently the causative agent/agents of CED is thought to be a waterborne toxicant. All farms that have experienced CED were using bore water. Elevated and/or imbalances of certain elements and/or compounds in the bore water, perhaps operating in synergy, may be directly affecting the sensor nervous tissues over the head and body of the fish. Interestingly, afflicted fish have been able to regenerate eroded tissues within 12 months after being placed in a surface water source.

6.4 Fish health management

The principal objective of fish health management strategies in intensive aquaculture operations is to maintain the health and well-being of stock, while optimising fish production. Key actions, which are critical to achieving this objective are:

- Take an active approach to managing the health of stock
- Maintain hygienic conditions
- Sterilise inlet water
- Guard against poor water quality
- Monitor water quality regularly
- Seek health certification for new stock
- Quarantine all new stock
- Minimise unnecessary stress
- Monitor health of stock regularly
- Feed fish an appropriate diet (composition, size amount).

6.4.1 Water supply treatment

A primary source of pathogens to intensive aquaculture facilities is the water supply. Both the quantity and quality of water used in aquaculture are critical to the well-being of fish under culture. A regular and abundant water supply is essential for any aquaculture operations. A number of water sources are currently being used for Murray cod farming including, surface waters (streams, rivers, lakes and dams), groundwaters (bore water) and domestic/urban waters (Ingram and Larkin 2000). Ideally these waters should be regular and reliable, free of pathogens, and free of pollutants (organic, industrial and urban) and consequently, regardless of the water source, some form of pre-treatment will be required before it is used in the culture system.

Untreated surface waters pose the greatest risk of pathogen contamination, especially if fish are present in the water supply. Treatment options may include combinations of the following:

- Mechanical screening (sand filters, carbon filters, screen filters) to remove particulate matter.
- Chemical treatment (chlorination followed by dechlorination and/or aging) to eliminate pathogens.
- Ozonisation to eliminate pathogens.

- UV sterilisation to eliminate pathogens.
- Elimination of fish from the water source (where possible).

Bore water is generally considered to be pathogen-free, however, since water quality varies widely from one source to another, groundwater requires thorough testing before use in aquaculture. Concentrations of some parameters (eg. iron, hardness, conductivity and dissolved oxygen) may render the water unsuitable for fish culture. Some form of pre-treatment may be required before it is suitable for fish.

Domestic waters provide a clean, hygienic alternative water source. However, the presence of high levels of chlorine compounds (used by councils to disinfect water for human consumption) necessitates the need to "age" or dechlorinate the water before use.

6.4.2 Managing new stock

Another important source of pathogens is new stock. To ensure that diseases are not introduced through restocking of aquaculture production facilities, all new stock, regardless of the source, must be quarantined. All aquaculture facilities, regardless of size, must have a quarantine facility that is separate from the rest of the facility. The quarantine system should be physically isolated from the other facilities (separate room or building). Water used in the quarantine system must be totally separated and must not be allowed to mix with the water of other culture systems within the facility. Equipment used in the quarantine area should not be used in other areas of the facility. Minimise traffic through the quarantine area and disinfectant footbaths should also be used at the entry/exist of the quarantine area. New stock should be quarantined for at least 2 weeks before introducing to the production facility. During that time, fish should be checked regularly for signs of disease, and should be given prophylactic treatments, such as salt and formalin baths, to eliminate external parasites which have previously been identified as a major health problem for farmed Murray cod (Fig. 6.1). Finally, the water quality conditions within the quarantine system may be different to that within the production system. Therefore, gradually acclimate new stock to production system water conditions during the quarantine period.

New stock should appear healthy at the time of delivery. The presence of fish that appear weak, underfed or unhealthy is a clear sign that the health problems may arise. Always purchase new stock from a reliable supplier, and always seek health certification. As an added measure of security, samples of fish should also be sent to a fish health specialist for disease screening.

6.4.3 Facility hygiene

A comprehensive cleaning and sanitising program should be established to maintain hygienic conditions within the facility. This program should include the treatment of all equipment that may be exposed to pathogens, including tanks, nets, buckets, meters, and floors. Sharing of equipment between different systems of the facility should be prohibited. Uneaten and spilled food and sick or dead fish should be immediately removed from culture systems. Regular cleaning of the floors to prevent the build-up of debris, including spilt feed, must be a regular activity. There are numerous cleaning and sanitising chemical compounds and agents. However, there is limited information on the performance/efficacy of these products, which makes it difficult to establish a quality control program (Flick 1998). Care should be taken in the use of these products, as they may be hazardous to humans, fish and the beneficial microorganisms within the systems (especially the biological filter). Some commonly used sanitisers include, chlorine solutions, iodine solutions, quaternary ammonium solutions, ultraviolet radiation, chlorine dioxide, phenols, alcohols and aldehydes (eg. formaldehyde) (Flick 1998).

Hygiene management should also extend to staff. Management practices may include provision of anti-bacterial soaps for washing hands, use of footbaths and disinfection of footwear and restrict visitation of people from other fish farms.

6.4.4 Dealing with fish health issues

6.4.4.1 Identifying the problem

Monitoring is an important part of early identification, management of disease problems. A decision support flowchart has been prepared to assist farmers in the identification of common health problems in intensive aquaculture systems (Fig.6.2).

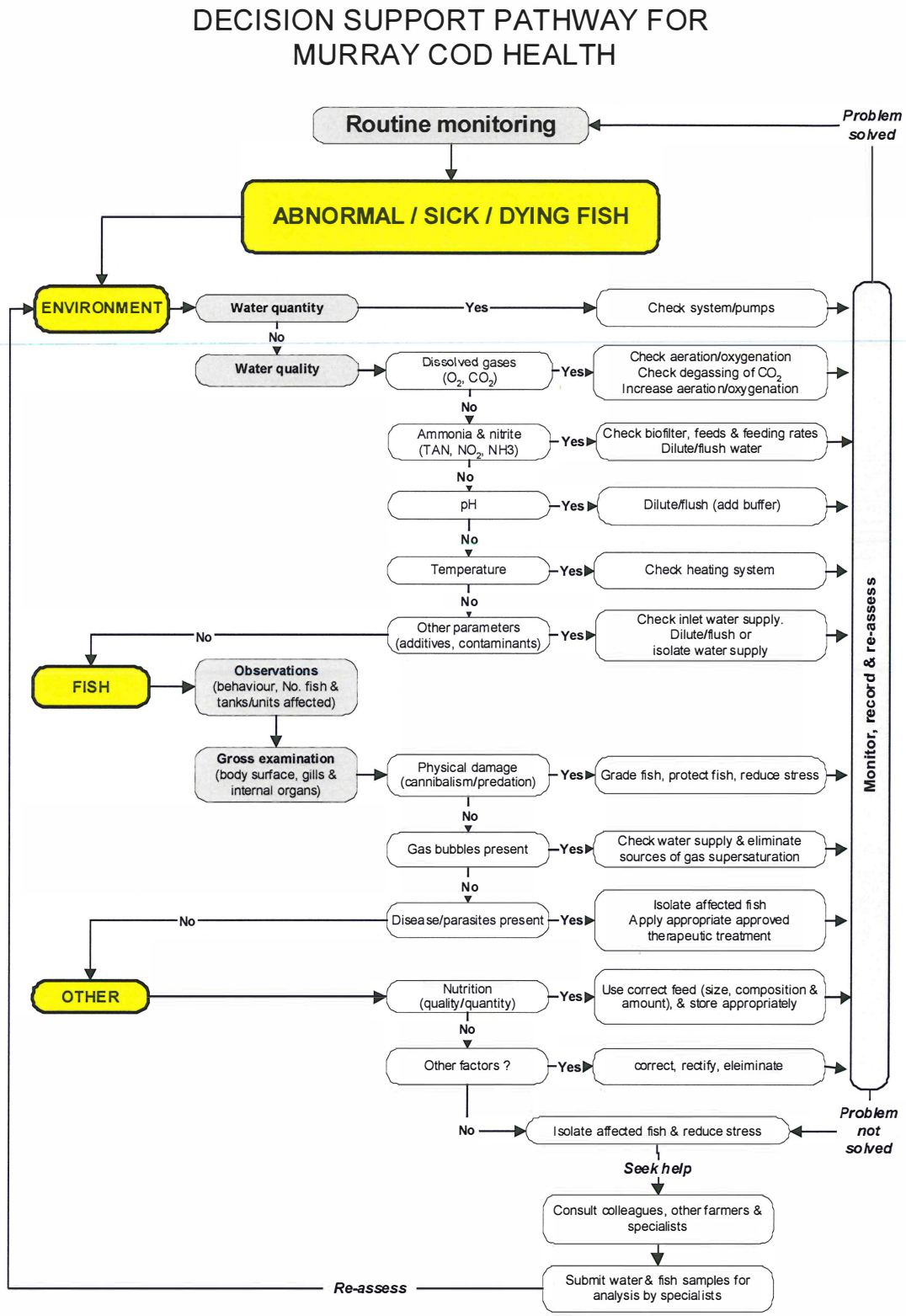

Fig. 6.2. Decision support flowchart for identification and management of major health problems in a Murray cod aquaculture facility

Changes in behaviour may be the first sign that fish are being stressed. These changes may include:

- reduced or cessation of feeding, lethargy, floating near the water surface and/or swimming with the head upwards, "flashing" or erratic swimming, and increased or laboured respiration (as indicated by "gasping", flaring of opercula or rapid opercula movement). Physical changes may become apparent, such as abnormal growths, lesions or discolouration on the body surface, loss of some scales and cloudiness of eyes. Fins may become tattered or eroded. The gills may become clogged with mucus and the filaments may appear swollen or fused together.

Water quality should always be checked to determine if key parameters, especially, DO, ammonia, nitrite, pH and temperature, have not departed from optimal or normal conditions. Visible examination of fresh wet preparations of skin mucus and gill tissue under a dissecting microscope or compound microscope may be sufficient to detect the presence of common parasitic infections (some fungi, protozoans and metazoans). However, identification of many pathogens, especially viruses and bacteria, requires the skill of a specialist using specialised analytical techniques. More detailed descriptions of the symptoms and diagnosis of fish diseases are found in fish health/disease texts including Rowland and Ingram (1991), Brown (1993), Herfort and Rawlin (1999), Noga (2000).

Immediate action in response to a health problem will assist in reducing the severity of the incident. Where possible eliminate the probable causative agent and reduce stress. Water quality problems may be alleviated by dilution or flushing with fresh water. Infected fish should be isolated from other stock within the facility. All equipment exposed to infected/contaminated fish and water should be disinfected. Treatment of infected fish with a therapeutic drug or chemical may ultimately be necessary.

6.4.5 Use of drugs and chemicals

Aquaculturists should only use chemicals and drugs that are either registered for use by the Australian Pesticides and Veterinary Medicines Authority (APVMA) (formerly the National Registration Authority (NRA)) (http://www.apvma.gov.au), covered by a APVMA Minor use Permit or are exempt from registration. Often, use of registered chemicals, and chemicals covered by minor use permits should be used under the direction of a registered veterinarian.

Under the Chemical and Veterinary Chemicals Code Act 1994, all agricultural and veterinary chemicals (as defined by the Act) must be registered by the APVMA before they can be supplied, sold or used in Australia. Chemicals and drugs use in aquaculture may also be governed by state legislation. In recent years, efforts have been made to facilitate the registration of selected chemicals for use in Aquaculture within Australia (Percival 2001). However, today very few chemicals have been registered for use in finfish culture in Australia (see http://www.apvma.gov.au).

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7 MARKETS FOR MURRAY COD AND AN INDUSTRY MARKETING PLAN

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CONTENTS

7.1 Introduction

Murray cod (*Maccullochella peelii peelii*), has been long recognised as an important species in recreational and commercial fisheries of inland south-eastern Australia. Aborigines fished for Murray cod, and throughout the late 1800's and early 1900's the species represented a significant portion of the commercial inland catch and were sold widely in markets in NSW, Victoria and South Australia (Kailola *et al.* 1993). During the 1970's and 1980's, artificial breeding methods were developed which allowed for the mass production of fingerlings for stocking to enhance recreational fisheries and conservation purposes (Ingram *et al.* 2004). More recently, private fish farms have been exploring the possibility of growing Murray cod to a table size for human consumption, and in the late 1990's farmed Murray cod started appearing in markets (Larkin and Ingram 2000).

Marketing aquaculture products is no different from marketing other agriculture products. Finance, cash flow, production and all other profit determining factors in aquaculture are intrinsically linked to marketing.

It is fair to say Australian primary producers have a history of "selling" products rather than marketing them. "Sellers" are very focussed on their production system and establish where a product will be sold only after it has been produced. "Marketers", on the other hand are customer focussed and are able to identify the needs of their customers. "Marketers" can provide the right product at the right price, in the right place, which attracts the customer to buy their product. The producer who develops a sound marketing strategy, and considers marketing as important as production will have a definite economic advantage over those who do not.

This paper addresses questions of marketing Murray cod more from an industry standpoint, rather than the position of individual farms. New entrants into the industry should focus on customer requirements, putting these needs at the forefront of all business planning and decision making. It must be noted however, that the Australian inland aquaculture industry needs to develop an industry wide marketing strategy, as consumers when viewed as an entity view aquaculture as an industry sector, and not as individual farms.

7.2 Industry Overview

7.2.1 Wild fishery

Historical aspects of the Murray cod fishery have intrinsically added to the value and mystical appeal associated with the species. In the mid- to late-1800's a large inland commercial fishery was developed, based mainly on the Murray and Murrumbidgee rivers (Rowland 1989). By 1883 the Murray River fisheries formed a considerable factor in the fish supply to Victoria and during this year more than 147 tons were sent to Melbourne from Moama (Cox 1884). In 1900, Murray cod accounted for 75% of the river fish available at the Melbourne market, the remainder being golden perch (Poole 1984).

Since the 1800's a well-established and high priced market for wild Murray cod had been developed in Australia based on fisheries in Victoria, New South Wales and South Australia (Kailola *et al.* 1993), and during the 1950's, when the fishery was at its peak, up to 311 tonne was landed (Fig. 7.1) (Ingram *et al.* 2004). However, since the 1950's the annual harvest of Murray cod declined dramatically, a trend attributed to a degradation of the aquatic environment related to modifications to rivers for hydro-electric, flood mitigation and irrigation schemes, pollution from agricultural, urban and industrial areas, overfishing, etc. (Cadwallader 1978; Cadwallader and Gooley 1984). As a result, in recent years more stringent management controls have been placed on both commercial and recreational fisheries to help protect the species in the wild. In particular, the minimum legal size is 50 cm (approx. 2-2.5 kg), and there is now a total ban on the take of Murray cod during the spawning season from 1st September to 30th November. Commercial fishing for Murray cod is banned in Queensland, Victoria and in NSW, and although commercial fishing is still allowed in SA, this fishery will be completely closed by July 2003 (Anon 2002).

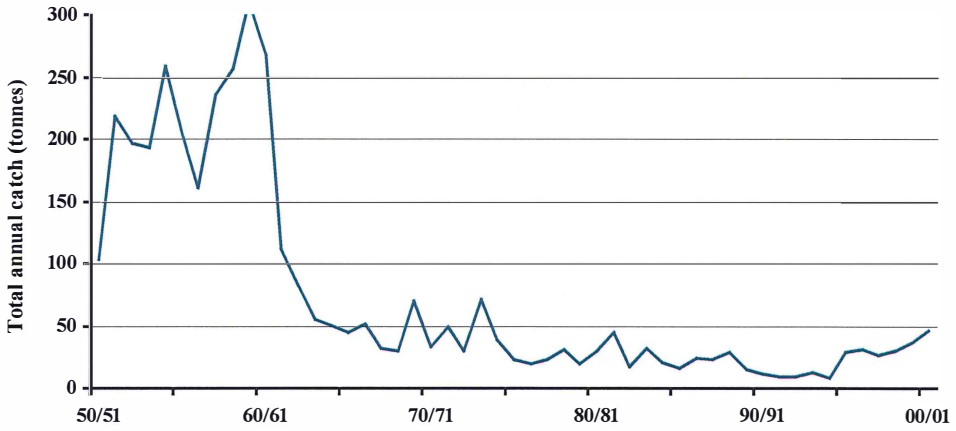

Fig. 7.1. The total annual commercial catch of Murray cod from 1950/51 to 2000/01 (After Ingram *et al.* 2004)

7.2.2 Aquaculture industry

During the 1970's and 1980's hatchery-based methods were developed for mass production of fingerlings (Ingram *et al.* 2004). During the 1990's private fish farms began investigating the growing of Murray cod for human consumption, and by the late 1990's farmed Murray cod were appearing in markets (Larkin and Ingram 2000). Large numbers of fingerlings are still being produced for stock enhancement programs, but increasingly fingerlings are being sold to other fish farms for grow-out (Ingram and Lawson 2004). Murray cod grow-out predominantly occurs in Vic., NSW and SA, and Murray cod are now routinely cultured for use in a range of markets (Table 7.1).

Sale prices and volumes have been closely observed in wholesale markets in both Sydney (Sydney Fish Market (SFM) (http://www.sydneyfishmarket.com.au/)) (Fig. 7.2, Fig. 7.3 and Fig. 7.4) and Melbourne (Melbourne Wholesale Fish Market (MWFM) (http://www.chsmith.com.au/fish-prices/melbourne.html)) (Fig. 7.5). At these markets, farmed and wild Murray cod are considered premium products when compared to other well-known farmed species (Fig. 7.4a). However, it should be noted that these prices are not truly representative for Murray cod, as higher prices may be obtained when marketing directly to distributors, retailers and restaurants. Nevertheless, these data are useful for comparison between species, which may be considered competitors to Murray cod, but does not necessarily represent the best-case scenario.

Table 7.1. Murray cod aquaculture products and markets

Facilities	Product (Size)	Markets
Hatcheries	*Post-larvae* (0.1g)	▪ Hatcheries.
	- live	▪ Grow-out facilities.
	Fingerlings (0.75-1.25 g)	▪ Pet and aquarium industry
	- live, non-weaned & weaned	▪ Stock enhancement - Private
		- Angling clubs
		- State governments.
		▪ Grow-out facilities.
		▪ Private fisheries (fish outs).
	Advanced fingerlings (1-5 g)	▪ Pet and aquarium industry.
	- live, weaned	▪ Grow-out facilities.
		▪ Private fisheries (fish outs).
Grow-out	*Juveniles/"stockers"* (75-300 g)	▪ Grow-out facilities.
facilities	- live, weaned	▪ Private fisheries (fish outs).
	Table fish (0.6 - 2 kg, up to 4 kg)	▪ Fish markets, wholesalers, distributors, retailers,
	- live	restaurants and consumers.
	- fresh, whole, chilled	▪ Private fisheries (fish outs).
	- gilled and gutted, chilled	
	- fillets, chilled	
	Brood fish (>0.6 kg)	▪ Hatcheries.
	- live	

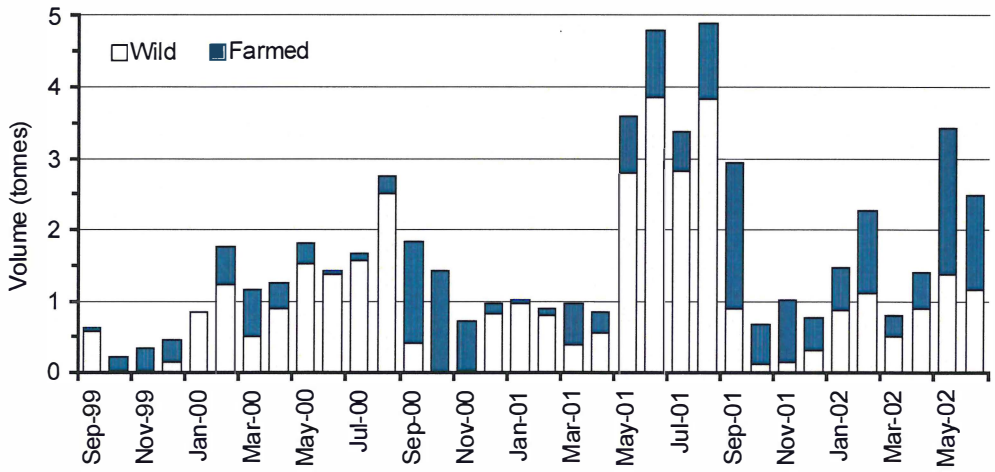

Fig. 7.2. Monthly volume of wild and farmed Murray cod sold at the Sydney Fish Market from September 1999 to June 2002.

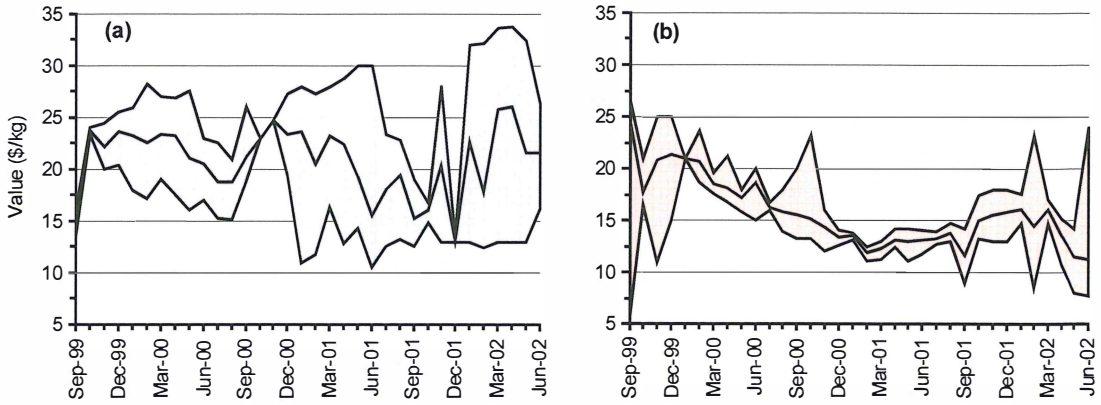

Fig. 7.3. Monthly minimum, maximum and average price paid for Murray cod sold at the Sydney Fish Market from September 1999 to June 2002. (a) Wild Murray cod. (b) Farmed Murray cod.

Approximately 50% of farmed Murray cod (by weight) sold at the SFM are from farms in Victoria, 30% from SA and the balance from NSW and Qld. More than 90% is sold as fresh fish on ice (Plate 3b), either whole or gilled and gutted, whereas less than 10% is sold as fillets. Prices paid for farmed Murray cod over the past three years have declined from a high of approximately $24.00/kg in late 1999 to a steady price over the last six months averaging approximately $13.50/kg (Fig. 7.3b, Fig. 7.4a). This reduction in price is not surprising as a similar trend has been observed in other intensively farmed fish that have undergone significant expansions in production (Asche *et al.* 2001). Nonetheless, prices for farmed Murray cod at the SFM and the MWFM are expected to remain at these levels while the quality remains high, and the volume does not significantly increase.

The volume of farmed Murray cod sold at the SFM is relatively low when compared to other aquaculture species (Fig. 7.4b), but volumes at both SFM and MWFM continue to grow slowly and other market sources report proportionately higher increases in volume over recent months.

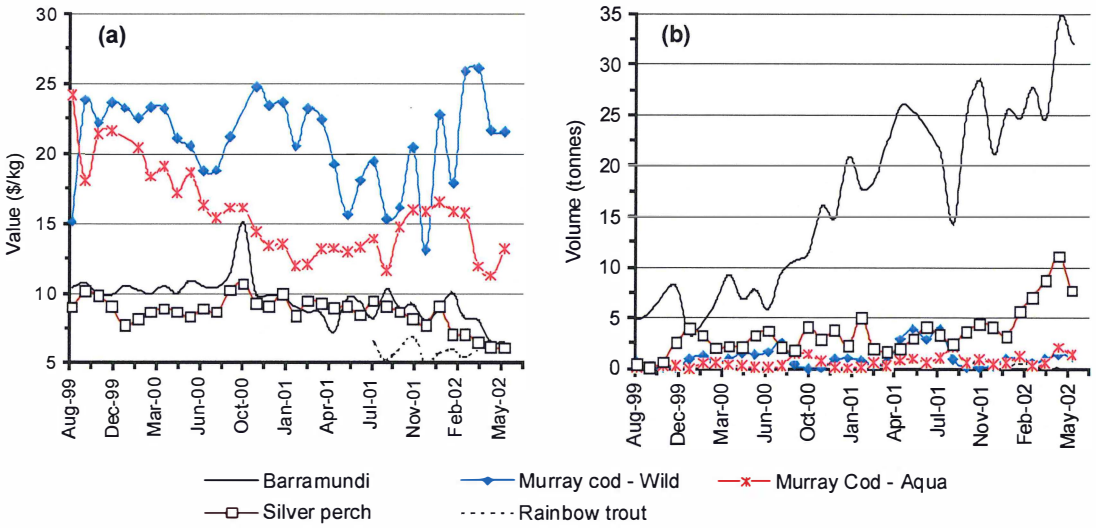

Fig. 7.4. (a) Value (monthly average) and, (b) volume (monthly total), of selected aquaculture species and Murray cod (wild and farmed) sold at the Sydney Fish Market from September 1999 to June 2002

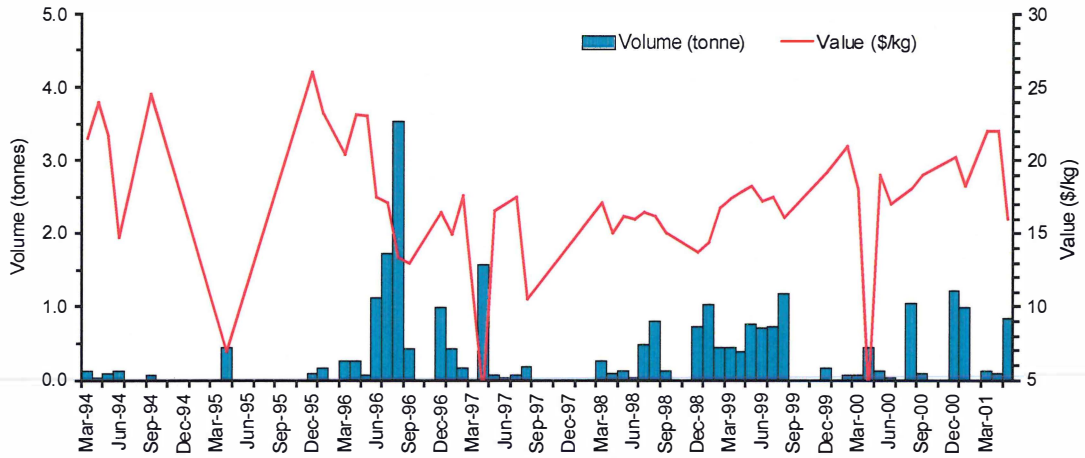

Fig. 7.5. Monthly volume and average monthly value of Murray cod sold at the Melbourne Wholesale Fish Market from March 1994 to May 2001

Farmed Murray cod has been well received in restaurants in both Melbourne and Sydney, where live fish, fresh chilled fish and fillets have been sold. Sales for both live and chilled Murray cod have generally been higher in Melbourne where marketing activity has probably been more intense. The marketing of Murray cod has until this point in time been undertaken by individual farmers, and the risk of collusion between a relatively small number of buyers to drive the price down, has been great. However, the buyer base is now spreading, and the risks have decreased.

While a fraction of farmed Murray cod is sold through the wholesale markets, farmers also employ other markets (see Section 7.3). Interviews with producers, fishmongers and retailers in Sydney and Melbourne indicate sales of live and chilled farmed Murray cod vary from about 600 to 1,200 kg per week in Melbourne and 500 to 1,000 kg per week in Sydney as at June 2002. Sales of live Murray cod represent between 20% to 50% of the total sales. The price paid for live Murray cod is significantly higher than that paid for the chilled fish (see Section 7.3), and though most fish farms prefer to supply steady weekly orders at a set price, it may vary slightly depending on the time of year, size of the fish, quality and quantity.

There is considerable scope for further sales of Murray cod within Australia, though the market is limited due to the relatively small population and resultant comparatively small level of demand. This will undoubtedly have an increasingly negative effect on prices and profitability for producers that focus exclusively on the domestic market. For this reason the development of export markets will need to be the long-term goal for the Australian Murray cod aquaculture industry if individual businesses are to sustain profits into the future. Exports of Murray cod have already occurred to many Asian countries, with the responses overall being positive (Stoney 2000; Anon 2001). Indeed, the development of some live, chilled and value-added Murray cod export markets is currently underway. Due to the confidential nature of these developments, they are unable to be accounted for in this paper, but potentially add significantly to the volume of the industry in the near future.

7.2.3 Comparisons between farmed and wild Murray cod

Wild Murray cod has highly variable volumes arriving at both the SFM and the MWFM, and the price varies considerably (see Ingram *et al.* 2004). Currently, the presence of farmed Murray cod at the markets appears to have had little effect on the price paid for wild Murray cod. However, at the SFM, there are apparent differences in the prices paid for farmed and wild-caught Murray cod (Fig. 7.3b). Indeed, buyers interviewed at both the SFM and the MWFM made a clear distinction between the wild and farmed fish. The reasons for this difference are not well understood, but may include:

Size of fish. Fish taken by commercial fisherman must be at leat 50 cm in length (approx. 2-2.5 kg) whereas farmed Murray cod are typically between 0.6 kg and 2 kg.

Appearance: Wild Murray cod typically have a pale green to creamy yellow background colour which becomes almost white on the ventral surface of the abdomen (Plate 1a & Plate 3a). This colouration is overlaid by an olive green to dark green mottled to marbled patterning. In contrast, both the background colouration and overlaying markings of Murray cod reared in intensive culture systems often become significantly darker in appearance (Plate 3a) and may be less attractive to some buyers.

Flesh texture and composition. The flesh of farmed Murray cod flakes well and is suitable for steaming, baking, grilling and frying, as whole fish or fillet. The flesh texture of farmed Murray cod is not as firm (softer) as that of wild Murray cod, which may be a reflection of the conditions in which they have grown. Murray cod is considered to be a non-fatty fish, as the lipid content of muscle is less than 2%, and a good source of omega 3 fatty acids (Mooney *et al.* 2001; De Silva *et al.* 2004).

Taste/flavour: There have been some incidences where farmed Murray cod have been downgraded at markets because of the presence of taints (muddy/earthy flavours) in the flesh. The presence of these taints can render a fish product unacceptable to consumers, and even the presence of a small amounts of tainted product at the market can severely reduce the market value of the product as a whole. The most prevalent off-flavours in farmed fish are caused by the compounds geosmin and 2-methylisoborneol. The primary sources of these compounds are certain species of blue-green algae and actinomycete fungi (Tucker 2000). Farmed freshwater fish can develop off-flavours associated with the culture conditions. However, these taints can be removed by purging prior to sale (see Section 7.4.2).

Up until the total closure of the NSW inland native fishery in September 2001, volumes of wild Murray cod sold at the SFM were considerably greater than for farmed Murray cod. However, with the pending closure of the sole remaining wild fishery in SA in July 2003, it is expected that this market will be taken up by the expanding Murray cod aquaculture industry.

7.3 Markets for Murray cod

For any primary industry to remain successful, it must offer a diverse range of products for consumption by the final purchaser. These products will need to be shaped and moulded to suit consumer requirements, which in turn may change over time. Primary producers from the more mature industries tend to supply wholesale or manufacturing companies who value add, or pass the appropriate product to the appropriate market channels. In many Australian aquaculture sectors, the producer takes responsibility for product value adding themselves. As such, resources and time must be allocated from the early stages of development by the farmer, in full realisation that he will be choosing those areas most profitable for the product. Further, in larger enterprises, several market channels may be required (often with one channel more profitable than another) to maintain a viable production unit where production matches supply in a reliable and overall profitable manner. The present outlets for farmed Murray cod are listed below with indicative price ranges and size ranges provided as a guide only.

The market alternatives listed below contain a synthesis of present activities and present some alternatives to, or enhancement of, present activities. These suggestions may aid in the future marketing of farmed Murray cod.

7.3.1 Live Murray cod

Live fish sales may be divided between domestic and export markets (Fig. 7.6 and Fig. 7.7). At first glance, both markets appear attractive to producers for both price and for ease of processing. The general concept is that fish are shipped to the appropriate restaurant or wholesaler alive and in good health, with lucrative gains promised. Also frequently promised by buyers, are large tonnages and continuing sales for all the farms production. Beware of such promises! There is a significant market for live fish in most major centres occupied by large numbers of Asian consumers. However, in Australia this market is limited, and must be handled carefully, or price and volume of sales may suffer. Worse still, the belief that Murray cod is a high quality and valued fish may be in jeopardy should varying qualities and quantities of fish arrive at individual markets in an uncoordinated manner.

As with all live fish there are issues associated with transport and maintaining Murray cod alive and in good condition at the wholesaler or distributor, and at the restaurant. While these issues are associated more with fish husbandry than with marketing, they do have an impact on the perception and quality of Murray cod on arrival to the restaurant. At present, there are only a few establishments one could consider adequate in terms of providing water quality suitable for holding any but the toughest of fish for any period of time. Indeed few restaurant owners monitor the water quality in tanks more than what may be achieved by a thermometer and removing dead fish. Fish that appear lively and healthy are attractive to clients of restaurants and will encourage sales and turnover for the establishment. On the other hand, fish that are in poor condition, or die before sale, will reduce profits for the establishment. Repeat purchases of any live fish that appear lethargic or stressed in the restaurant display tank, or die before sale, are rare.

It is important that the retailers and owners of restaurants purchasing Murray cod be educated in basic husbandry and water quality maintenance techniques. At present, Murray cod are being placed in display tanks along with other species (Barramundi, silver perch, etc), or are placed in tanks by themselves at low stocking densities. It is not appropriate to stock Murray cod in display tanks with other species as optimal conditions for maintenance will vary between species. Many factors, such as lighting, the point of entry of influent water in the tanks and stocking densities, have been found to dramatically affect the survival and well being of Murray cod in the display tanks of retailers and restaurants. The first step in educating these people may take the form of something as simple as a leaflet, detailing holding requirements for maintaining the health of Murray cod, including lighting, water quality (temperature, pH, oxygen, and salt requirements) and stocking densities. These people are interested to learn, and are genuinely disappointed when fish die, or are in poor health in their tanks.

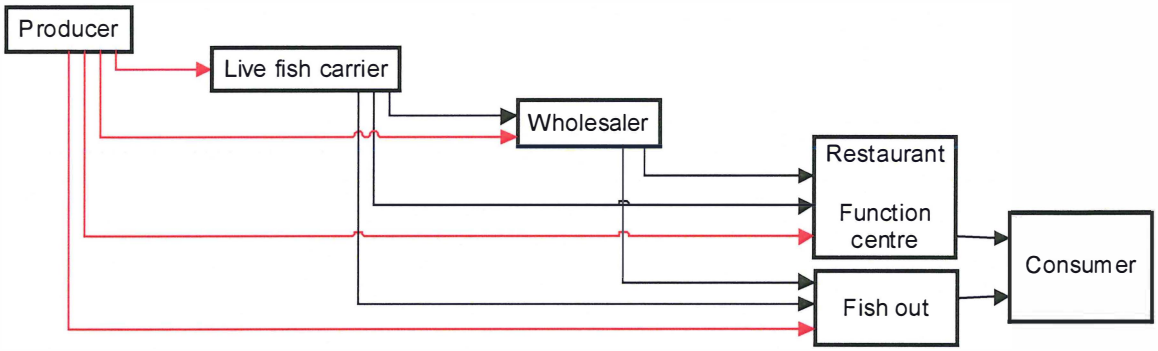

Fig. 7.6. Distribution model for domestic live Murray cod markets

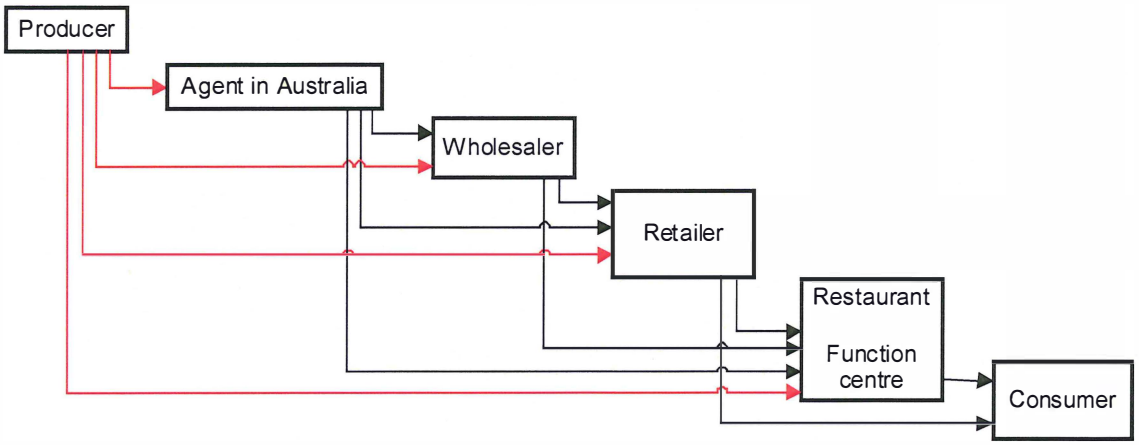

Fig. 7.7. Distribution model for live fish export to Asian destinations.

While maintaining the health and appearance of Murray cod in display tanks is important, consideration should also be given to the overall presentation. Improvements to presentation may be as simple as altering lighting in the tank, changing background colour within the tank, or increasing the frequency of cleaning of the tank itself to ensure clear viewing of the fish. These factors together should assist to increase sales share of the live fish market.

7.3.1.1 Domestic markets for live Murray cod

Live fish are sold, almost exclusively directly to the retail and seafood market sector, targeting tourists and communities of Asian ethnic origin, all of whom are generally prepared to pay premium prices for such a product. At present, there are two definite size ranges of Murray cod required in the live fish domestic market:

(a) 0.6 kg to 1 kg are required when selling directly to distributors supplying restaurants and members of the general public, and/or when selling directly to restaurants. The reasons given for requirement of these sizes are:

- Fish are sold per piece to the consumer, and per kilo from the farm, so a reasonable sized fish that is not too large is a good alternative, providing a good margin for profit.
- Fish are usually consumed by two or more people with rice and plates of different meats. If the fish is too small there is not enough for all to receive a portion, and if it is too large there is the risk of wastage.

(b) 1 kg to 2 kg are required for function centres and restaurants hosting functions. The reason for the requirement of larger fish is associated with the number of people per table at the function. As many as eight to twelve people per table will consume several different types of food. The fish is an important centrepiece, and a larger fish is required for all at the table to have a portion.

It is important to stress that when the customer asks for a certain size of Murray cod that a particular size be supplied. There are alternative species for functions centres, restaurants and general domestic use, and most of these have an established, reliable name for supply and quality. A higher price is paid for live Murray cod than most species, with the exception of perhaps coral trout (*Plectropomus* spp.) and some other reef species. To maintain the fish in this price bracket the quality of Murray cod must be kept at a premium standard.

The introduction of the GST caused much consternation amongst the ranks of live fish retailers and wholesalers with many not fully understanding the laws and requirements of the Australian Tax Office. The situation has improved marginally of recent times as most have improved their knowledge and acceptance of the tax regime. There is still some confusion relating to the requirement for GST to be charged on some live seafood products, and not on others, such as crayfish.

Murray cod that are sold live are mostly between 0.6 kg and 2 kg (Plate 2d & Plate 3b), with smaller numbers of larger fish (up to 4,000g) also being sold. At present reliable live fish sales represent between 200 kg and 300 kg per week in Sydney and 250 kg to 500 kg per week in Melbourne (personal communication with producers). These fish are fetching approximately $12.00/kg to $17.00/kg farm gate and selling for $18.00/kg to $30.00/kg retail. Large live fish (3-4 kg) have fetched up to $45/kg in some Sydney restaurants (O'Sullivan 2002). The price at restaurants varies greatly, with most selling per piece, and the price per plate differing with the standard plate price of the restaurant. Some concern has been expressed regarding the maintenance of the above prices with increasing volume of product appearing on the market. Suggestions to aid the maintenance of price and build volume of the market are presented in Section 7.5.

Small volumes of Murray cod are sold into Adelaide and the Gold Coast on an irregular basis. Large, irregular fluctuations in demand occur from time to time in all markets and may be associated with major events, functions, Asian weddings, large Asian tourist groups or delegations, or the lack of supply from competing species. These irregular fluctuations are generally not sustained on a week-to-week basis but must be supplied on demand to build Murray cod as a good alternative species, or replace the existing species in these markets. More reliable peaks in demand occur in the weeks leading up to Christmas, the Chinese New Year and Easter. Once again, to establish Murray cod in these markets it is important to satiate these peaks in demand for Murray cod as they occur.

An additional component of the domestic live fish market is private fisheries. These are privately owned waters (lakes, dams, ponds etc) which have been stocked with fish that customers pay a fee to angle. Some operate as catch-and-release fisheries, while others are fish outs at which anglers keep their catch. Private fisheries for trout are already well established across south eastern Australia, and private native fish fisheries are being developed. As there are few places where native fish may be caught the initial step in establishing a fee fishing operation is to determine customer needs. In addition to the size of Murray cod required, the cost of amenities and staff to monitor and collect money for the fish caught must also be included. A major benefit is the premium price paid for fish, which may be as much as double that paid for whole chilled fish sold through wholesale channels.

7.3.1.2 Export markets for live Murray cod

The export market for live Murray cod is primarily to Asian centres where the main demand is believed to exist. At present exports of live Murray cod vary in frequency and volume, from samples only one week to several hundred kilos the next. This scale of variation is not unusual in export markets, when both the production systems and the market are developing.

Singapore and Hong Kong are seen as two of the more progressive seafood markets in Asia. Several importers in these countries have expressed strong interest in working with reliable Australian suppliers to develop markets for Murray cod. However, these markets face strong competition from other suppliers of seafood products, and are price sensitive. Murray cod is seen as a premium, high quality freshwater fish and as such will not fetch the prices demanded of selected marine fish (in particular reef fish).

The amount of reef fish imported annually to Hong Kong has been estimated at between 30,000 to 35,000 mt, with a total wholesale value of US$490 million (McGilvray and Chan 2002). While freshwater fish volumes and price are below this estimate, it still forms a substantial portion of the market. Despite the enormous volume and monetary value, the live fish trade is not well understood and what little information is available is out of date, particularly since fish prices from 1997 or before are still being quoted. In fact, fish prices tumbled by nearly 50% following the onset of the 1997 Asian financial crises. Although most Asian economies are recovering the prices for live fish remain low compared to the prices before the financial crises.

Australia is renowned as a supplier of high value, wild caught seafood products, such as abalone and rock lobster. With the ongoing development of aquaculture, our key export markets need to be made aware of Murray cod and the value it represents as a high quality product. Murray cod has been compared favourably with Grouper and Mandarin fish in China (Chinese buyers, *pers com.*). Moreover, importers view Australia as a source of high quality product that is produced in a healthy and safe environment. This presents a considerable opportunity for the promotion of Australian Murray cod, branded as a safe, quality seafood product. Such claims must be substantiated to customers in these markets and the importance of adequate quality assurance and production systems cannot be overemphasised.

The strongest global demand for finfish, particularly in Asia, is for marine species with a white or red flesh. The flesh should be relatively firm, not disintegrate when cooked, and have a sweet and clean flavour with minimal bones. The preferred size of Murray cod for these markets varies, but is generally between 0.6-1.0 kg. These consumer preferences fit nicely with Murray cod as a culture species. Murray cod has all the physical attributes mentioned above, and the required size may be reached within a twelve-month growing period.

The development of export markets will require considerable investment in promoting and educating buyers and end-consumers about species for which they are unfamiliar and do not initially prefer. The Murray cod industry and/or individual producers may need to be prepared to incur some costs to establish market access, until their 'new' species or products effectively infiltrate the market. Often this requires that suppliers make free samples available for targeted 'in-market' product launches and promotions.

Producers considering marketing Murray cod to Asian destinations must decide where they intend to enter the market (see Fig. 7.7). There is some debate whether to use agents in Australia, or endeavour to sell to wholesalers or retailers in the country of final destination. For small to medium producers of seafood the ease of payment and the day to day expenditures of energy and finance required to succeed in export, make the agent in Australia an attractive option. Other seafood exporters have established reliable communication

and distribution channels with wholesalers and retailers in Asia and are comfortable they are able to control the many issues associated with export from a remote location.

In general, the retailers control the market distribution and when purchasing fish from wholesalers take a profit of 24% to 35%. Restaurants are the main end users and mark the price up 100% to 150% on the purchase price from the retailer (McGilvray and Chan 2002). Murray cod is presently selling for approximately AUS$20.00/kg to AUS$25.00/kg in Hong Kong, where it is competing effectively with other freshwater species such as mandarin fish (*Siniperca* spp.). To continue growing the volume and price, aggressive marketing activities are required.

Additional information on fish markets in Hong Kong, Japan, Singapore and Taiwan, and marketing Murray cod in these markets, is provided in Stoney (2000) and Anon (2001). Advice on how to establish and enter export markets is available through various government agencies, including the commonwealth government agency, Austrade. This agency has offices located in various countries around the world and provides support and services to Australian companies including market research, and identifying and introducing potential importers. Contact the Austrade hotline on: 132878 or visit http//www.austrade.gov.au.

7.3.2 Chilled Murray cod

While the sale of live fish is regarded as the most lucrative market, the chilled fish market is thought to have potential to absorb considerably larger volumes of Murray cod. Chilled fish products include, whole fish not gilled and not gutted (Plate 3b), whole fish that are gilled and gutted and fillets (skin on and skin off). Dressed weight (gilled and gutted) of Murray cod is approximately 80%-85% of whole weight, while fillet weight is about 40% of the whole weight (50% of dressed weight). As described by Yearsley *et al.* (1999), the fillets of Murray cod are generally white, moderately deep, short and gently tapering in shape, and the integument is greyish above and white below. Producers may choose to sell directly to wholesale markets, retail outlets, distributors, restaurants or function centres, or to the public at the farm gate (Fig. 7.8). This chilled fish sector may also include Asian participants, and is capable of substantial growth in volume with proper market promotion and/or lower prices.

7.3.2.1 Direct sales to consumers

Direct sale of chilled Murray cod to consumers is a good place to start if supplies of products are small or irregular. Good sales may be expected if the farm is located within a short distance from large population centres. Where possible orders should be taken prior to purchase for both live and chilled fish to avoid inefficiencies associated with multiple small sales at irregular and often inconvenient times. Pre-ordering also reduces the time wasted waiting for a customer and enables processing and clean up operations to be kept efficient, organised and hygienic. Prices for Murray cod sold direct to the public have varied from $15.00/kg to $35.00/kg.

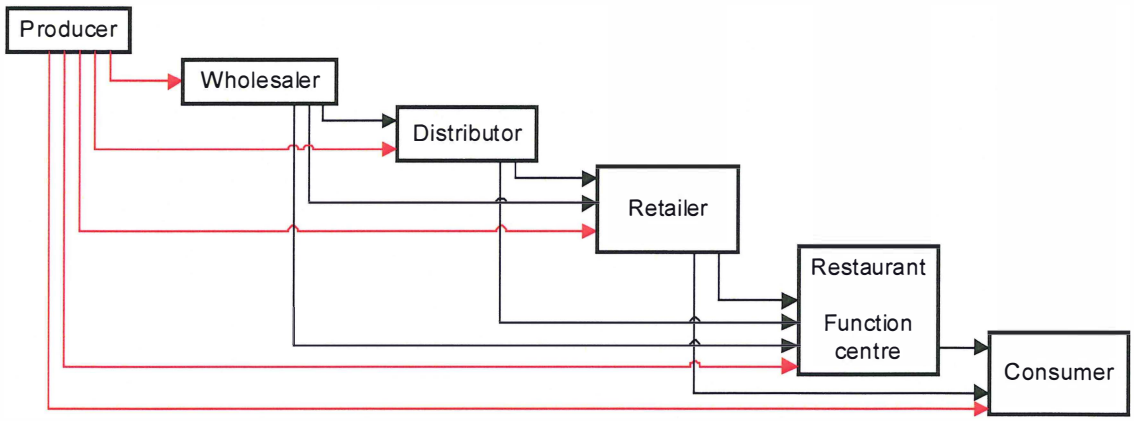

Fig. 7.8. Distribution model for chilled Murray cod to domestic markets.

7.3.2.2 Sales to restaurants and function centres

The greatest increase in seafood consumption in Australia is occurring outside the home. Penetration into the broader community is therefore likely to follow the promotion of aquaculture products within the restaurant and catering industries. The trendsetting capacity of these industries is not to be underestimated. One method of developing awareness of Murray cod to the broader public as a high quality fish that is available all year round is to establish the fish on menus at high quality restaurants. To determine what price a restaurant is prepared or able to pay for a particular product, the supplier must have a working knowledge of how the price per plate/serving is arrived at. The following figures are indicative and intended as a guide only:

$$Price\ per\ plate = \frac{food\ costs}{\%\ mark\ up} \quad \text{..... Equation 1}$$

$$Food\ costs = portion\ cost + vegetables,\ salads,\ sauces\ etc \quad \text{..... Equation 2}$$

$$Portion\ cost = whole\ fish\ price \times \frac{kg\ per\ portion}{return\ on\ fillet} \quad \text{..... Equation 3}$$

Working with these assumptions, if a restaurant is asking $20.00 per plate, and has a 30% mark up, the food costs will be $6.00 per plate (Equation 1.). The vegetable portion of any food cost may vary between $1.00 and $3.00 per plate. At best the farmer can expect to charge $5.00 per portion to the restaurant (Equation 2.). Portion size requirements for most restaurants vary between 150g and 200g, and the return as skin on wing off, ribs out fillets for Murray cod is around 40% (expressed as a fraction 0.4). Given the above figures and allowing for no costs associated with filleting, transport to the restaurant and/or any wholesaler or retailers, the price per kilo for whole fish to the farmer on a $20.00 plate with a 150g portion of Murray cod is $13.33 (Equation 3). For a 200g fillet the return to the farmer for whole fish per kilo is $10.00. This example illustrates that small variations in any of the parameters in the equations above can mean the difference between profit and loss to the farmer.

7.3.2.3 Sales to retail outlets

Present market channels, such as the domestic Asian sector and the white tablecloth restaurants, have a limited capacity to absorb high priced fish. However, there are possibilities for increases in sales in the retail sectors. Retail outlets sell fish directly to the consumer and include supermarkets and seafood outlets. Marketing activities targeted toward these sectors should be in the more affluent retail outlets. In recent years, supermarkets have changed their attitude toward seafood with stores accepting different products dependent on the demographics of the clientele. Supermarket outlets have diverse buying strategies. Some will deal primarily with large wholesalers, but will accept product from producers in niche markets or regions not seen across the whole chain of stores. Others are willing to accept products on a trial basis, and usually encourage promotion of that product at set times within nominated stores. It is likely the requirements of supermarkets will vary with time and between the different chains of stores. As sufficient volumes of Murray cod become available for consumption, dialogue between members of the industry and the supermarket chains is required to develop a strategy to gain access to selected stores. The presentation and processes required by the supermarkets are stringent and require time and resources for implementation. Supermarkets are capable of selling large volumes of seafood, however pricing is extremely competitive, and supply must be guaranteed.

There are significant volumes of Murray cod presently sold through retail outlets in markets (such as the Queen Victoria Market in Melbourne and Paddy's Market in Sydney), and shopping centres in areas such as Footscray and Springvale in Melbourne and Haymarket in Sydney. With the appropriate marketing and pricing strategies in place, these outlets will grow in numbers and volume of product sold.

7.3.2.4 Sales to wholesalers and distributors

Wholesalers by and sell large quantities of fish to distributors, retailers and restaurants. Distributors on the other hand, also tend to buy large quantities of product, but then sell in smaller quantities to their clients,

which are mostly restaurants and function centres. Sales to wholesalers and distributors generally require larger levels of production. While this may be appropriate for larger producers, smaller producers may be able to access these markets through the establishment and use of co-operatives.

7.4 Factors influencing the Murray cod market

Fish markets in general are influenced by many factors. These factors range from local fluctuations relating to the market segment occupied by the product, to the global economic situation. While seafood producers, retailers and wholesalers can have little effect on the macro-economy of the globe, the immediate factors affecting the markets and marketing of Murray cod are still in a developmental phase, and may be influenced by the actions of those in the industry over the next few years.

It is important to stress that competition in the marketplace for any fish species is not only other fish but also other meats that may be purchased at a similar or cheaper price per plate. Many of these meats are from larger, mature primary industry sectors, such as chicken, lamb and beef, which are supported by massive marketing budgets. These meats establish prices per plate that are used to compare with those paid for fish. Once again, there is little that can be done to change the effect of these meats on the market sector occupied by fish. However the fish market sector is increasing in Australia, and will continue to increase with the correct marketing strategies in place.

7.4.1 Market prices and perceptions

In the broader community there is general lack of awareness of the availability and quality of Murray cod for the household table. Options to firstly increase awareness of Murray cod to the broader community, then to assure consumers Murray cod is available in good quantity and quality will be addressed in the establishment of Marketing Plans and is outlined in Section 7.5.

Viewing the market of Murray cod from the purchasers' point of view, one factor affecting the volume of Murray cod sold is the perception that Murray cod is a high priced fish to buy. The commonly compared species include farmed barramundi and salmon (see Fig. 7.4), and wild golden perch which are usually cheaper than Murray cod in the market. Prices exceeding $10.00/kg limit repeat purchasers of farmed Murray cod to the top restaurants, some Asian consumers, and those few affluent consumers who are well educated in the value of quality fish. Following extensive seafood trade interviews estimates varying between 70% and 90% of the Murray cod sold in the domestic market is consumed by the Asian section of the community, 10% to 20% by top restaurants and the remainder by non-Asian consumers. Options for increasing sales while maintaining the price of Murray cod are discussed in Section 7.5.

It is important to note that the majority of Murray cod is presently sold in the live or whole chilled form. As previously detailed (see Section 7.3.2) Murray cod fillets represent approximately 40% of the total weight of the fish and when sold the difference between Murray cod and cheaper fish magnifies with each step in the chain.

7.4.2 Murray Cod quality and quantity in the marketplace

Until this point in time, farmed Murray cod supplied primarily to markets in Sydney, Melbourne and various export destinations has been of varying quality and volume (eg see Fig. 7.2, Fig. 7.5 and Fig. 7.3).

7.4.2.1 Quantity

In the process of developing a marketing plan it is important to recognise Murray cod must be promoted to attract new consumers and in addition to building on the present base. Marketing is required to be conducted in a more direct fashion than merely placing the product on the wholesale market floor and accepting the price offered. The buyers attending the wholesale markets have limited budgets and may have a multitude of choices offered on the day of sale. The volume of Murray cod, the volume of competing species and the overall volume of seafood available at wholesale markets have a direct influence on the price paid for Murray cod on the day of sale.

Buyers are presently purchasing cultured Murray cod with little distinction between brands of the different growers. Growers as a group, or industry should therefore beware of supplying the market with excessive volumes of product on any one day. Options to avoid downward pressures on price and to alternate market

channels are discussed in Section 7.5.

7.4.2.2 Quality

Health considerations are expected to play an increasing role in consumer choices for seafood and the role of omega 3 fatty acids in consumer health has been well documented (Sinclair 1993; Rice 1999; Hamazaki and Okuyama 2001). CSIRO and Deakin University analysis indicate Murray cod is a good source of omega 3 fatty acids (Mooney *et al.* 2001; De Silva *et al.* 2004). In fish, the location of fat deposition differs depending on species and fish size (Sheridan 1998). De Silva *et al.* (2004) showed that when Murray cod are fed diets containing in excess of 17% lipid, excess fat was deposited in the viscera rather than the muscle. De Silva *et al.* (2004) further suggested that Murray cod should be considered to be a non-fatty fish as the lipid content of muscle of both farmed and wild Murray cod was less than 2%. Nevertheless, comments have been received from seafood traders to the effect that there is a lot of fat (presumably visceral) in cultured Murray cod, most of which is thrown away making the remaining edible flesh more expensive.

It is of paramount importance that presentation is of the highest standard when Murray cod is sold through retail outlets. Fish appearance, especially the appearance of the eyes, fins and body colouration, may have a significant bearing on market sales. One observation from several fishmongers interviewed indicated that direct contact with ice to the eye of the fish turns the eye white in colour. This white colour indicates to some buyers the fish is not fresh or is less attractive to the consumer, and the volume of Murray cod sales deteriorate. Fish presented with tattered, eroded or split fins may also fetch lower prices.

Murray cod reared in intensive recirculation systems usually become much darker in appearance than wild fish and as such may be less attractive to some buyers (see Section 7.2.3) (see Plate3a). Some purging processes and the culture, or "finishing-off", of Murray cod in cages or ponds is reported to produce fish with a colouration more typical of wild fish (Ingram 2004).

In addition to presentation, buyers at the fishmonger counter makes his or her choice based on many factors, such as price, the recommendation of the fishmonger, the variety of competing species, and the size of the available portion. Farmed product may be portrayed in a consistent appealing fashion in the fishmonger's window thereby providing a competitive edge to other products available at the time of purchase, and a sense of familiarity to the consumer. Fishmongers interviewed during the present study suggested marketable fish that are less attractive due to their appearance should be sold as fillets rather than sold as live or whole chilled fish and risk deflating market prices.

There have been reports of "earthy" or "off flavours" in farmed Murray cod presented to markets in both Sydney and Melbourne (see 7.2.3). As with other cultured freshwater fish, such as silver perch (Rowland 1995; Ruello 1999) and channel catfish (Tucker and van der Ploeg 1991; van der Ploeg and Tucker 1993; Tucker 2000), earthy taints in the flesh of marketed Murray cod must be avoided. Purging of fish is the generally accepted method for removal of earthy taints, however the techniques for adequate purging are yet to be determined. Investigations into purging techniques have been undertaken for other species (Engle *et al.* 1992; Tucker 2000), but research into the purging of Murray cod is required. The process required to fully remove off-flavours from fish by purging may include considerations of water temperature, chemical composition of the purging water, size of fish and species of fish.

The effect of negative perceptions resulting from poor quality fish cannot be overestimated in the short term, and often has lasting and serious detrimental effects on the market in the long term.

7.4.2.3 Value adding fresh chilled Murray cod products

Some of the main barriers to increased market share for fresh chilled Murray cod are associated with the lack of value adding of the product. Because of the perishable nature of fresh fish, quality barriers to satisfying the best paying and freshness conscious consumers increase with the time between the time of processing and the time of sale. Thus increased demand for fresh quality fish increases the need for closer relationships between primary producers, primary processors, and the distributors of fresh consumer packed products. The role of primary producer and primary processor may be carried out on-farm, or at a dedicated fish processing plant.

Value adding of seafood products aims to gain a greater market share, and/or to increase the price paid for the product. This is achieved by increasing the shelf life of the product and/or improving and enhancing the

appearance of the product to the consumer.

Some of the value adding options which may be considered for Murray cod products include:

1. *Branding of the whole fish, fillets, and processed products* (including whole smoked, smoked fillets and pates). Branding may take the form of distinctive logos, names and/or colours on packaging and opercula or other tags attached to individual fish or fish products. As a general rule when choosing a name for the product avoid using long names, names similar to those already being used and recognised, and names unrelated to the product.

2. *Processing Murray cod into various forms,* including:
   - *Fillets*. These may take the form of skin on or skin off, ribs in or ribs out, wing on or wing off. The various forms are dictated by the demands of the market/consumer, and should be priced considering the weight lost from the whole fish, processing time and labour required, as well as any specialised packaging or transport requirements.
   - *Smoked products*. Head on gilled and gutted (HOGG), fillets and some by-products have been smoked and may provide alternative markets to chilled, frozen and live fish markets. Further value adding of smoked products includes pates and dips, which utilise varying amounts of the primary smoked product. More work is required in this area to determine the economic viability resulting from various smoked Murray cod products. Some preliminary trials of smoked Murray cod indicate the flesh smokes well in the "hot smoked" form, but is not suitable for "cold smoked" products.
   - *Frozen products*. As a general rule, frozen fish sells for less than chilled or live fish. The early indications are that Murray cod will freeze well, and do not produce the "watery" and "grainy" texture associated with many fish on thawing. However, the end price is yet to be determined for HOGG or fillets of Murray cod.
3. *Packaging*. The various forms of processed Murray cod and the different market channels may require different forms of packaging. For example, some supermarket chains are interested in modified atmosphere packaging for fresh fillets. The gases and membranes involved may vary from species to species and from the different forms of processed products. Other examples include vacuum packaged and "blister packs". These are considered good alternatives for smoked product. When considering packaging the costs of materials, labour and infrastructure must be balanced against increased market share (hence increased volume sold from the farm) and increased prices gained for the various products.

7.5 Developing a marketing plan for Murray cod

A marketing plan should be developed for an individual enterprise, as part of its overall business plan. This plan can take many different forms and should be developed in a format that is most useful to those expected to implement it. However, the following elements are a guide as to what should be incorporated into a marketing plan for any one enterprise considering cultured Murray cod. The information presented below draws largely on concepts and ideas presented for the marketing of inland aquaculture products from integrated agri-aquaculture systems (Stoney 2003).

7.5.1 Factors in developing a marketing plan

7.5.1.1 Vision:

that has been developed for the business (during the business planning stage) should be at the forefront during this process. The marketing strategy contributes to achieving this overall vision for the business in question.

7.5.1.2 Market Research:

a planned approach to identify potential customers and to define their needs (including product specifications). A "SWOT" analysis is a useful tool for analysing the information identified in this process, which considers the Strengths, Weaknesses, Opportunities & Threats of the potential markets for Murray cod. Internet Websites, such as the SFM (http://www.sydneyfishmarket.com.au), and the MWFM (http://www.chsmith.com.au/fish-prices/melbourne.html), are useful sources of information for determining broad market trends (species, prices, volume traded etc), and various popular media publications, such as 'The Age Good Food Guide", are useful tools to identify specific potential market segments. Developing a

network with other producers and operators within a defined supply chain can also facilitate access to other important sources of market information.

7.5.1.3 Key market variables:

The following key market variables need to be considered when developing marketing goals and formulating a marketing plan:

Product:
is the item being offered for sale, which includes its physical aspects such as taste, appearance, size, weight and quantity, and other product attributes such as after sales service. Other examples can include quality assurance accreditation (disease, food safety, environmental management etc), packaging, and value adding of your product. Indeed product specification, which is backed up by independently accredited/certified protocols such as HACCP, ISO1400, is likely to be essential in the future to maintain and/or increase market access. In short, it is critical to get the product 'right', according to consumer needs and specifications, in particular its quality and consistency of supply. Diversification by developing different product lines, particularly through value-adding and portion control (supplying different sized/shaped portions of the whole product), also increases interest from a wider range of potential buyers.

Price:
is usually determined by how much the customer is prepared to pay, matched with how much the producer is prepared to accept (partly determined by production costs and associated target profit margin). A marketing plan should incorporate a competitor analysis, to take into account world supply patterns in addition to local supply patterns for farmed Murray cod. Pricing of aquaculture products specifically must be competitive with other seafoods (wild caught and cultured), but also to a large extent with other agricultural commodities which consumers will readily substitute for fish if prices are too high. Price setting by a producer may be a useful marketing tool to achieving better farm gate prices. The advantage of this situation is that buyers can know costs and estimate profit margins in advance. For the food service sector in general, knowing prices well in advance is invaluable to costing portions and the projected financial return from the menu that is set.

Place:
where the customer wants to purchase the product (which typically must be convenient to, or readily accessible by them). Issues such as distribution, freight and storage logistics and location of outlets are important factors to consider. This will involve segmenting the market into different target audiences. To reduce risk, most producers try to diversify and develop a number of different market outlets for farmed Murray cod. Considerable ongoing investment is required in maintaining and growing existing markets, but also in identifying and developing new markets for farmed Murray cod.

Promotion:
how effectively the marketer lets the customer know farmed Murray cod will meet their needs. It is critical that these messages are realistic and do not over emphasise features that the company or product cannot deliver. It is about getting the right message to the right people and may include personal contact, internet, in-store promotions, conventional advertising, product brochures, and targeted trade shows. Building strong 'whole-of-supply-chain' relationships is very important for promoting your product, as this will ensure you are receiving direct feedback from consumers. In this context the 'branding' of farmed Murray cod in Australia is likely to be critical to achieving significant market access, particularly for export purposes. This is most relevant presently due to the heightened recognition by consumers and authorities around the world of the need for accredited/certified disease-free, safe food products. Producers can certainly gain competitive advantage in the marketplace by implementing relevant Best Practice standards and associated Codes of Practice, which becomes a valuable promotional tool for the industry. Some of the more established inland aquaculture producers in Australia have found that using a direct mail-out, followed by individual visits, has been effective for them in promoting their products. Producers have also found that educating potential buyers about specific products is critical. This has been achieved by providing product information, such as consumer handling guides (which can have a major effect on the end quality of the product), and free samples to wholesalers, retailers and consumers. Participation in trade and food shows is also a useful vehicle to educate buyers and the general public about aquaculture products.

Implementation:
is about developing the processes to make it all happen.

Plan of Action:
who, how and by when? A decision making process for achieving the required sales results.

Processes:
to determine how things are done in the organisation to ensure the business maintains its customer focus. This will include procedures for dealing with customer inquiries and monitoring their satisfaction.

Resourcing:
adequate budget, adequately skilled people and time allocated to make it happen. The business must have people readily accessible and in constant contact with the buyers.

Positioning:
the strategies needed to get a share of the available market, which is particularly important when there is competition between suppliers. Techniques such as pricing, promotion, and special services can improve and develop a more positive perception about Murray cod, over its competitors.

There appears to be significant opportunities and benefits in developing a centralised marketing network and distribution system in Australia, particularly for Murray cod producers. This network would require producers to adhere to strict quality parameters, and to ensure that the production integrity of all suppliers is maintained. This is particularly important if a product brand is developed for this centralised or networked supply.

Review and evaluation:
is about ongoing assessment through feedback loops linked into the supply chain, of what you are doing, including evaluating the success of your plan and addressing the question as to whether it is achieving your strategic vision and business objectives. When measuring the 'success' of your plan, it is necessary to consider not only financial outcomes but also family and social impacts. Environmental sustainability outcomes are increasingly important key drivers of market access and commercial success in aquaculture and agriculture enterprises. The ability to integrate both disciplines within production landscape therefore enables producers to legitimately market their produce within an environmentally sustainable context, and thus gain a competitive advantage over producers that do not comply with such rigid standards.

7.5.2 A marketing plan for the Murray cod aquaculture industry

Australia has a small number of high quality aquatic species suitable for commercial inland aquaculture production, including both introduced and endemic species. Some of these species are currently being produced and are well established in the market place (eg, silver perch, rainbow trout and barramundi), while others are deemed to be new and developing species (eg Murray cod), which have yet to establish themselves as commercially viable products.

Either way, planning for the growth and marketing of the increasing supply of Australian inland aquaculture products for both domestic and export consumption is seen as essential. Such planning is required in the first instance at an appropriate level, including national, state, regional and/or species specific, and needs to take account of the diversity in structure and operational philosophy of the businesses it is intending to assist. This is particularly important for a new and developing sector, such as farmed Murray cod, with its inherent production characteristics and potential competitive market advantages.

An industry-wide marketing plan should start with a broad national strategy relevant to all producers, which might then ultimately filter down to a more detailed plan, such as a joint marketing strategy for a specific region or farm. The more established and experienced producers of Murray cod will need to be the key drivers of this planning process as such a plan will require their experienced input and must be applicable to them if it is to be implemented.

Developing export markets for species that are relatively 'unfamiliar' to consumers can be a very costly exercise. It is logical that the Australian industry works together with Government to prepare an export market development strategy, which will ensure that Australian suppliers and exporters do not directly

compete against each other in these markets. The perception that Australia is a 'clean and green' supplier should be the basis of any industry marketing strategy, provided that the production and quality assurance systems are in place to substantiate such claims. Australia's proven track record in the supply of high value marine, wild capture species, such as abalone and rock lobster, can also be built on.

Part of this planning process could include the development of a recognisable product brand, compatible with the needs of consumers, as a means of securing a market profile. Such an exercise would require careful consideration of producers' ability to maintain consistent supply, substantiation of the image or claims being portrayed by the brand, and the logistics of physically labelling or identifying fresh, unprocessed or processed Murray cod.

The ability of producers to support branded Murray cod in the market place in an effective and efficient manner will be critical to ultimate success. Part of this requirement dictates the need for the establishment of viable production and marketing networks, together with the development, adoption and implementation of appropriate Best Practice Management Guidelines and associated Codes of Practice for quality assurance and sustainability purposes.

The Murray cod aquaculture industry, if it is to respond to the needs of the market, is to supply consistent quantities of safe, high quality products, which has a competitive advantage over competing products. The challenge for this industry is to substantiate claims of quality and safety, and to develop reliable lines of supply.

7.6 Conclusions

Commercial harvesting of wild Murray cod has been in existence since the late 1800's, and until recently has been considered to be a small but highly valued industry, with fish often fetching premium prices in various markets. This industry, however, will cease with the closure of the last remaining wild fishery in SA. Development of the Murray cod aquaculture industry will provide an alternative source of Murray cod for the market. Murray cod farmers are now striving to establish a niche in seafood markets, but are faced with issues associated with perception and acceptance of what is considered to be a relatively new and unproven product.

As a "new" product, farmed Murray cod has excited considerable interest from buyers and producers alike. Domestic consumption has grown rapidly and export of product is now occurring. At present the approach to marketing is largely *ad-hoc*, conducted by individual farmers and targeted toward local niche markets or the larger and usually more competitive markets in the major cities. As the volume of farmed Murray cod grows, the requirement for coordinated, targeted marketing becomes more important. The establishment and development of opportunities for domestic and export markets for farmed Murray cod requires a thorough exploration of the market alternatives and the complex factors influencing seafood markets. Development of a marketing plan is required for the industry to expand.

Market development of new products often follow a similar pattern:

1. Developing awareness by consumers.
2. Increasing availability of the new product.
3. Changing attitudes toward the new product.
4. Changing preferences for consumer products.
5. Developing new consumption patterns.

Currently the Murray cod aquaculture industry lies somewhere within the mix of the first three developmental steps. With coordinated and focussed marketing activities the industry should now endeavour to move towards development of steps four and five.

7.7 Acknowledgments

This article was compiled with data up-to-date to 30 June 2002, supplied by several seafood industry sources. Many people contributed to this article, however the contributions made by the following people in particular are gratefully acknowledged. Mr Malcolm Nottage, 3 Rivers Fish Supply (Kangarilla, SA), Mr

Robert Clementson, Sydney Fish Market (Sydney, NSW), Mr Roger Camm, Australian Aquaculture Products (Euroa, Vic), Mr Tom Birdsall, Racovolis Amalgamated Seafoods (Melbourne Wholesale Fish Market, Vic) and Mr Tony Djeng, Watken Specialty Seafoods (Melbourne, Vic). The authors also wish to thank the SFM and the MWFW for permission to present data from their respective internet webpages.

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8 THE ECONOMICS OF PARTIAL AND INTENSIVE MURRAY COD RECIRCULATION AQUACULTURE SYSTEMS

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CONTENTS

8.1 Introduction

Recirculation (intensive) aquaculture systems (RAS) are a relatively new technology for holding and growing a wide variety of fresh water and marine finfish in Australia. These systems come in an array of capacities and efficiencies (Larkin 2000). Through the effective management of production variables, recirculation technology offers relatively more independence from the external environment. This translates to an increased level of control, which can provide a basis for improved risk management.

This paper investigates the financial and economic efficiencies of two small scale partial recirculation systems (1 tonne and 5 tonne) which are integrated into traditional farming practices and three intensive aquaculture enterprises (25 tonne, 50 tonne and 100 tonne) growing Murray cod (*Maccullochelli peelli peelli*).

Best practice industry data is used (growth, FCR, mortality, equipment and running costs) in conjunction with AQUAFARMER™[1] feasibility software to determine the relationship between key bio-economic

[1] AQUAFARMER *(V2.1)* is propriety software developed by Fisheries Victoria. It is specifically designed to analyse recirculation aquaculture efficiency and viability. It is a 10 year accounts simulator that creates farm scenarios based on bio-economic inputs.

variables such as the sale price of the product, FCR, stocking density and growth.

8.2 Advantages of recirculation aquaculture systems

Recirculation systems represent relatively new technology with a wide variation in system design and quality. Through the effective management of production variables, recirculation technology may offer relative independence from the external environment.

Recirculation aquaculture systems are receiving increasing interest in intensive fish culture operations as technological advances in closed systems technology. Small business ventures in particular are attracted to enclosed and modular recirculation systems. The closed system offers several advantages, including:

- water and heat conservation
- waste management control
- fish health control
- stock management
- site flexibility
- increase stocking density
- out-of-season production.

Improvements in feed formulations, nutrition, water chemistry, disease prevention and treatment, and selection of species with economically desirable traits could well lead to continuous production improvements. The identification of species with economically desirable traits include those which have the following important aspects:

- Established markets
- High value
- Tolerance to wide range of water quality
- High stocking densities
- Feed on pelleted food
- Efficient food conversion ratio (FCR)
- Seedstock readily available.

8.3 Management risk

The level of control inherent in recirculation systems can provide a basis for improved risk management. The trade off, of course, is a necessary increase in technology dependence and associated expense and the expertise to manage it. Low cost, small-scale entry into the industry is often recognised as a means of limiting financial exposure while gaining valuable experience. This approach is now widespread and yet it can lead to complex equipment retro-fitting, higher production risk margins and technological shortcuts that may be costly in the medium to long-term.

While there may be a cost incentive to de-construct recirculation systems into component parts by adding or subtracting from established designs, in practice this should not be considered lightly. It must be recognised that water re-use systems involve complex water chemistry in a finely tuned balance and that deviation from proven designs increases the fish farmer's risks significantly.

In best practice recirculation systems more than 90% of the water is recirculated through a series of purpose built biological and mechanical filtration systems so that only a fraction of the water is actually consumed. The importance of the biological filtration sub-system cannot be overemphasised. A recirculation system in effect grows two organisms – fish and bacterial culture resident in the bio filter. Nitrifying bacteria that convert ammonia and nitrite, which are toxic to fish, to less toxic form of nitrogen is critical to the overall success of the system. The bio filter must be constantly managed to ensure optimum performance and hence optimum fish growth.

A bio-economic simulation of recirculation aquaculture was carried out for Tilapia by Kazmierczak and Caffey (1996). The simulation carried out an optimisation sequence for:

- 7 levels of biological filter efficiency (BE) ranging from 1 to 0.7,
- 4 levels of mechanical efficiency - solid removal efficiency (SRE) ranging from 1 to 0.25,
- 3 levels of dietary protein (20, 30, 40% dietary protein), and
- 4 levels of stocking density ranging from 0.07 g per litre to 0.13 g per litre.

The bio-economic simulation model suggested that movements in biological filtration efficiency (BE) has a far greater impact on net returns than combinations of the other three variables. As biological filter efficiency falls, time to harvest increases at an increasing rate, and net returns decreases at an increasing rate. Kazmierczak and Caffey (1996) further indicated that:

- higher stocking levels may lead to economic failure
- economic trade offs between feed quality (dietary protein) and stocking occur over a narrow range
- a higher degree of management expertise is required in optimising the system to maximise returns.

The study concluded that the efficiency of biological filtration was critical to the success of the venture. Biological filtration efficiency had lower limits whereby alternative management of other parts of the system may not compensate and the system may fail.

One of the greatest problems associated with this technology is that while emerging technical blockages may be overcome technically they may not be economically solvable. As producers intensify their aquacultural activities the margin for management error becomes more acute as the more intense bio feedbacks occur. The inevitable link between stocking densities, necessary to cover the higher fixed and variable costs associated with closed systems as compared to open or semi closed systems, and margins of error suggests that economic success is more allusive.

The quality of investment decision making is related to the degree of pre start-up business planning which requires a comprehensive assessment of production costs, markets and a sound identification of risk. The lack of expertise and knowledge of both production and economic variables will increase risk in venture failure. This is particularly relevant where traditional agricultural operators look to diversify into aquaculture without access to the appropriate skills. Intensive recirculation aquaculture systems demand a high degree of technical dependency and the expertise to manage it.

8.4 Murray cod

8.4.1 Introduction

Murray cod is becoming a premium species for aquaculture in Australia, especially Victoria (Ingram *et al.* 2004). Recent industry experience suggests that stocking densities of over 100 kg/m^3 could be obtained with little mortality and a grow-out period to plate size (500-1,000g) in 10 months (Ingram and Lawson 2004). This was despite previously held views that the species were territorial and aggressive and therefore unsuitable to high density stocking.

Murray cod is one of the largest freshwater fish in the world and is endemic to the Murray-Darling river system (Harris and Rowland 1996). It is valued for recreational, commercial and conservation purposes. In the wild they attain maturity at 4-5 years weighing between 2.5 and 5 kg and can grow up to 113 kg (Harris and Rowland 1996). A female can produce around 3,200-7,600 eggs/kg (Rowland 1998).

Murray cod is a highly sought after as a table fish (with a high protein content) and up until recently has supported a lucrative but otherwise relatively small commercial fishery for many decades (Ingram *et al.* 2004). However, the distribution and abundance of the species have declined markedly since European settlement, and commercial fisheries production is now restricted to small quantities of Murray cod being landed from within South Australia only. New South Wales has recently banned commercial fishing for Murray cod.

Over the last 10-20 years, techniques have been developed that enable routine, large-scale hatchery production of Murray cod (Ingram *et al.* 2004). This technology however, is largely limited to the seasonal production of fry and small fingerlings between 30-50 mm, or 0.5-1.5 g. Private and state government fish hatcheries in Victoria, New South Wales and Queensland annually produce fish for stocking public and

private waterways for both recreational and conservation.

Existing strategies for the grow-out of Murray cod involve harvesting pond-reared fry, acclimatising them to tank conditions and then weaning the fry onto artificial diets for the purpose of over winter production in specially designed tanks enclosed in insulated sheds (Ingram and Lawson 2004). Current farming methods are producing market size fish (>500 g) in 10 months. This usually takes 2-3 years in the wild.

Growth patterns of Murray cod indicate that fast growers can reach marketable size within 10 months while the slower growers can reach this size in 20 months (Ingram 2004). Typical growth curves of Murray cod reared in a recirculation system under commercial production conditions are shown in Fig. 8.1. The growth data used to produce these results have been estimated from industry sources Primary Industries Research Victoria (PIRVic) Murray cod growth modelling and should be taken as a guide only. Seedstock are available seasonally only (November – March), but within that season there may be several intakes of seedstock to the production system. Two intakes of seedstock (November and January) and three growth cohorts (slow growers, average growers and fast growers) were used in the present model (Fig. 8.2). Based on the growth curves presented, the grow-out period is considered to be up to 20 months with minimum product weight being 500g. Under this scenario, full production is not achieved until the 3rd financial year after installation (Fig. 8.2).

8.4.2 Marketing

There is considerable interest in farmed Murray cod (both plate size and larger). Producers, wholesalers and retailers see Murray cod as an ideal species to satisfy a significant latent domestic and export demand. Such a demand is in part driven by the premium and associated ongoing demand placed by Asian markets in cultured grouper, and the perception that Murray cod are a species which could be equally well marketed throughout Asia. A recent preliminary market appraisal of Murray cod in Taiwan, Hong Kong, Singapore and Japan has indicated positive market response (Stoney 2000; Anon 2001). On product quality parameters, Murray cod was considered highly competitive with other premium freshwater finfish present in those export markets.

At the present time, up to 50% of the annual Australian pond production of Murray cod fry has been laid down for grow-out purposes (Ingram and Lawson 2004). With the increasing demand for seedstock, both the numbers of fish and actual producers involved is likely to increase significantly over the next five years.

Prices for farmed Murray cod on the domestic market vary according to size, grade and market. Average whole (HOGG) prices on the Melbourne Wholesale Fish Market (MWFM) (http://www.chsmith.com.au/fish-prices/melbourne.html) and Sydney Fish Market (SFM) (http://www.sydneyfishmarket.com.au/) have fluctuated over the past 8 years as the markets adjust to quality and quantity (see Larkin *et al.* 2004). In general prices paid for farmed Murray cod have declined from a high of approximately $24/kg in late 1999 to as low as $8/kg in 2002. The low prices observed in 2002 were in part due to the dumping of inferior quality product. The forgoing analysis has assumed a price of $15.00 per kilo for good quality 500g fish.

8.5 Economic analysis of different farm sizes

8.5.1 Introduction

Business planning that is attuned to the complex interplay of bio-economic variables will have an overriding influence on the viability of an aquaculture venture. Best practice industry data is used (growth, FCR, mortality, equipment and running costs) in conjunction with AQUAFARMER™ feasibility software to determine the relationship with key bio-economic variables such as the sale price of the product, FCR, stocking density and growth. This article was compiled with data obtained from commercial Murray cod aquaculture operations collected during 2002.

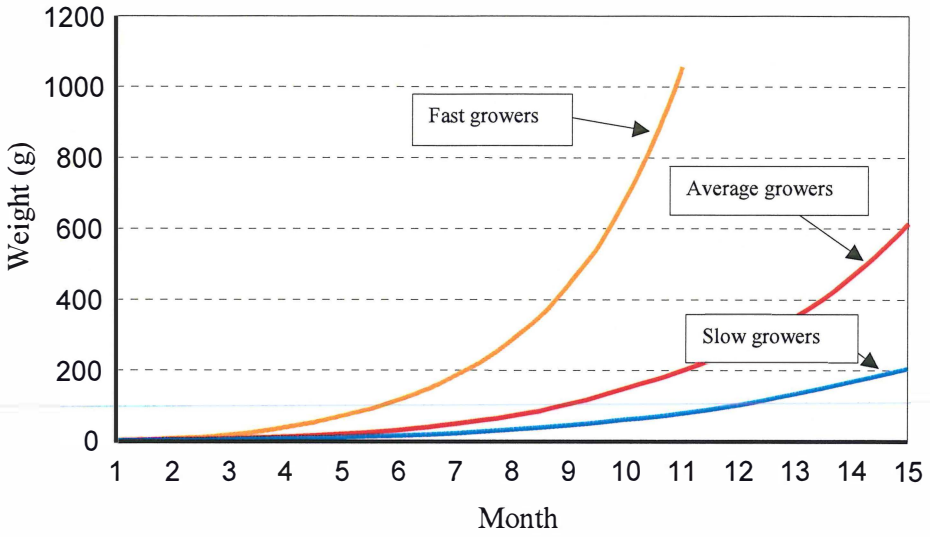

Fig. 8.1. Growth curves of Murray cod in a recirculation aquaculture system

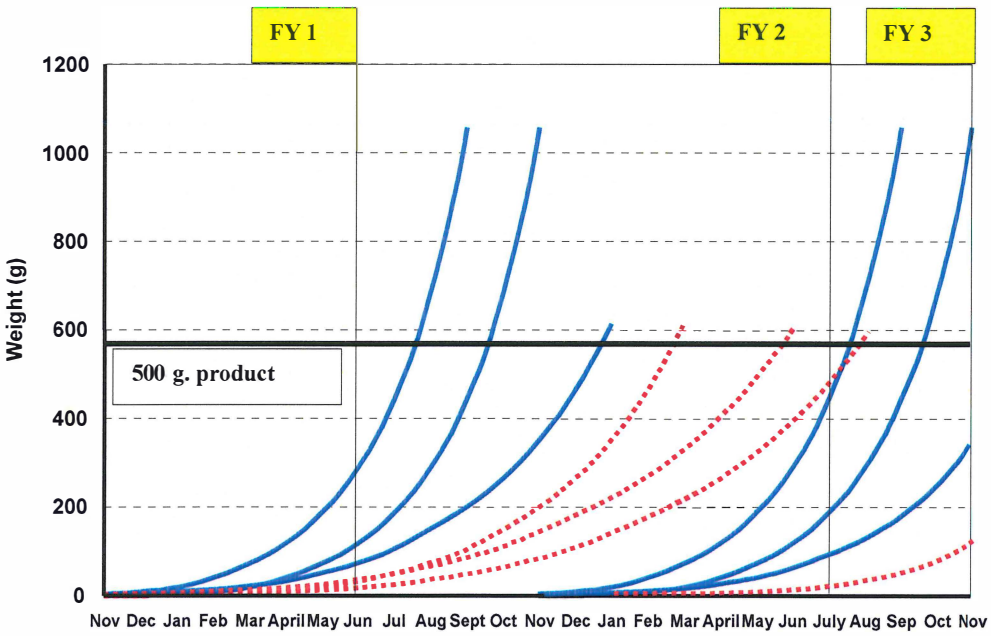

Fig. 8.2. Growth cohorts of Murray cod stocked into a RAS in November and January (FY = Financial Year) (Solid line = Growth curves for November intake of seedstock. Dotted line = Growth curves for January intake of seedstock)

In developing AQUAFARMER™, particular attention was focused on generating feasibility reports that reveal the critical link between key bio-economic variables and financial performance. AQUAFARMER™ provides a model or platform that can be used to run different case studies or scenarios based on the use of different key inputs that can present best case/worse case scenarios. The scale of production is a critical element in determining the costs associated with producing fish. The cost per fish will decline as scale increases.

8.5.2 Farm descriptions

The current level of production for Murray cod aquaculture in recirculation systems ranges from 1 to 100 tonne/annum/farm (Ingram and Lawson 2004). Therefore, the following farming structures were selected to compare scales of production in terms of tonnes produced (whole fish) per annum.

8.5.2.1 Hobby farm

A 1 tonne farm (equipment capital outlay of approximately $35,000) and delivering a modest surplus of $3,000 after expenses but no wages.

8.5.2.2 Small partial recirculation agri-aquaculture integrated farm

A 5 tonne farm (equipment capital outlay of approximately $150,000) and delivering a surplus of $15,000 to cover labour and/or expansion plans.

8.5.2.3 Small intensive recirculation farm

A 25 tonne farm (equipment capital outlay of approximately $400,000). This type of venture is best suited to an on-farm 2 person venture with possible other income supplementing family income (salaries of $75,000) plus a modest surplus generated each year.

8.5.2.4 Medium recirculation farm

A 50 tonne venture where fish farming is the only activity of the enterprise. Land is assumed to be in place but the cost of specialised buildings is assumed to be part of the capital set-up cost (equipment capital outlay of approximately $800,000). The salary component of $130,000 covers two professional staff plus employment on-costs.

8.5.2.5 Large recirculation farm

A 100 tonne large scale specialised single venture where fish farming is the only activity of the enterprise. Land is assumed to be in place but the cost of specialised buildings is assumed to be part of the capital set-up cost (equipment capital outlay of approximately $1.5 million). The salary component of $210,000 covers four professional staff plus employment on-costs.

8.5.3 Common base data

In order to compare and contrast the five different scales of production, farm cost and bio-economic parameters were standardised as much as possible. These common cost items and bio-economic parameters are detailed in Table 8.1.

8.5.4 Scale specific capital expenditure

Each scale of farm will require appropriate capital expenditure to meet the pressures of producing increasing tonnages (Fig. 8.3). These costs are one-off costs that occur in capital set-up. It is assumed that no new capital equipment will be required to be replaced over a 10 year period. Depreciation is calculated on a straight-line basis and shows up in the profit and loss accounts.

It is assumed that land does not have to be purchased but brought into the project as an asset by the farmer and that there is no borrowing's. Capital start up costs also includes the initial purchase of fingerlings to be grown out.

Table 8.1. Assumptions used in farm models

Description	Value
Price (HOGG)	$15.00
Cost of fingerlings	$0.60
Cost of Water [1]	$200 per ML
Electricity Cost per Kilo of Fish	$0.10 per Kg
Cost of Weaning Tanks $ per cubic metre	$450
Cost of Grow out Tanks $ per cubic metre	$250
Tank Volume (Weaning)	2 cubic metres
Tank Volume (Grow out)	20 cubic metres
Licences (aquaculture, water etc.)	$2,000
Feed Costs	$1.80 per kg
Property Tax	$3,000
Biological Parameters	
Stocking Density	100 kg/m^3
FCR	2.3 to 1.2 over 20 months
Mortality (Month 1 and 2)	10%
Grow out period	20 months
Financial	
Discount Rate	5%
Corporate Tax	36%
Stock Insurance (% of turnover)	4%

1. 1 and 5 tonne integrated agri-aquaculture farms have nil water costs associated with fish farm as costs have already been paid for by principle farming activity

Recirculation technology consists of the following capital goods items, however smaller farms may not employ all of the components:

- Mechanical and Biological Filtration Systems
- Fractionator
- Degassing Equipment
- Ozone and Oxygen Equipment
- Ultra violet Equipment
- Pumps
- Monitoring and Control Systems
- Backup Equipment
- Plumbing.

The building required to house the fish farm is a purpose built insulated design to ensure that temperatures are kept as stable as possible and that systems maintenance and harvesting are optimally designed into the floor plan.

8.5.5 Scale specific running costs

There are a number of running cost variables that will change as the scale of the farm increases due to the increasing production and accompanying administrative and maintenance costs etc. These estimates are detailed in Table 8.2. Based on these values, the composition of running costs (mean values) for a Murray cod operation is presented in Fig. 8.4. The highest running cost is labour (27%), followed by feed (26%) and seedstock (16%).

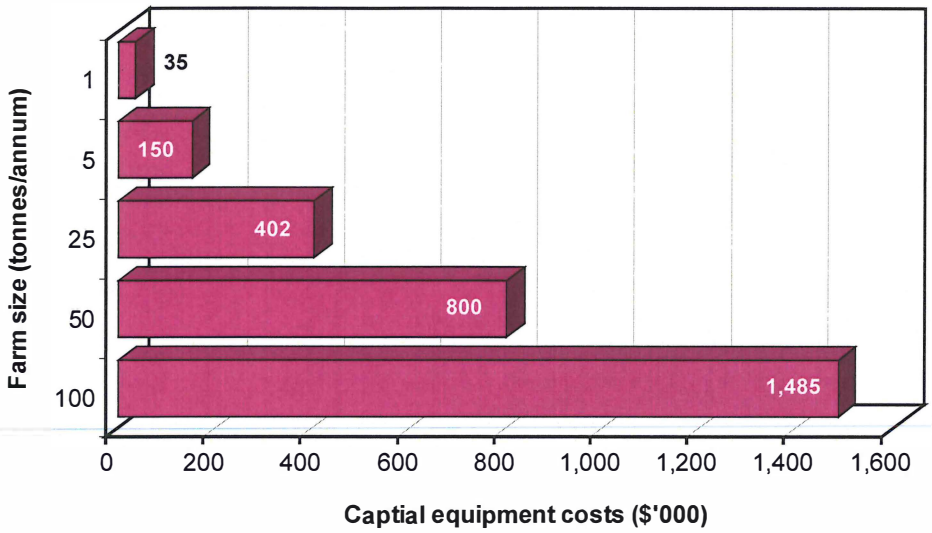

Fig. 8.3. Capital equipment costs for initial set-up of Murray cod recirculation systems

Currently there are no commercially available Murray cod feeds. Instead the industry used diets formulated for other aquaculture species, such as salmonids (trout and salmon) and barramundi. The feed conversion ratio (FCR) has an important impact on running costs (feed represents around 20% of total running costs at FCR of 1.2) as more food is required to achieve the same weight gain. The increase in FCR could be due to many reasons, including:

- Poor feed quality
- Poor feed management
- Poor water quality and oxygenation
- Poor husbandry techniques
- Stocking regime.

Table 8.2. Farm capital, set-up and running costs

Description	Farm size				
	1 Tonne	5 Tonne	25 Tonne	50 Tonne	100 Tonne
Equipment	$35,000	$150,000	$402,000	$800,000	$1,485,000
Capital and Running (Y0&Y1)	$40,000	$160,000	$520,000	$1,000,000	$2,000,000
Seedstock	$1,800	$8,500	$40,000	$82,000	$168,000
Standing Capacity	0.45 tonne	3 tonne	14 tonne	28 tonne	58 tonne
Growout tanks [1]	4	2	7	15	29
Labour [2]	$3,000	$15,000	$75,000	$130,000	$210,000
Electricity	$900	$6,100	$26,300	$66,000	$114,000
Feed	$1,600	$15,300	$72,400	$148,400	$305,600
Administration	$350	$1,000	$5,000	$10,000	$15,000
Marketing	-	$2,000	$5,000	$10,000	$15,000
Fuel	$500	$2,000	$3,000	$5,000	$10,000
Repairs & maintenance	$500	$1,000	$5,000	$20,000	$50,000
Insurance (B & E)	-	$200	$5,000	$10,000	$15,000

1. Growout tanks are 10 cubic metres in 1 tonne operation.
2. Labour costs for the 1 tonne and 5 tonne farms have been extracted from Net Profit after tax while the labour costs of the larger integrated systems have been included in operating costs.

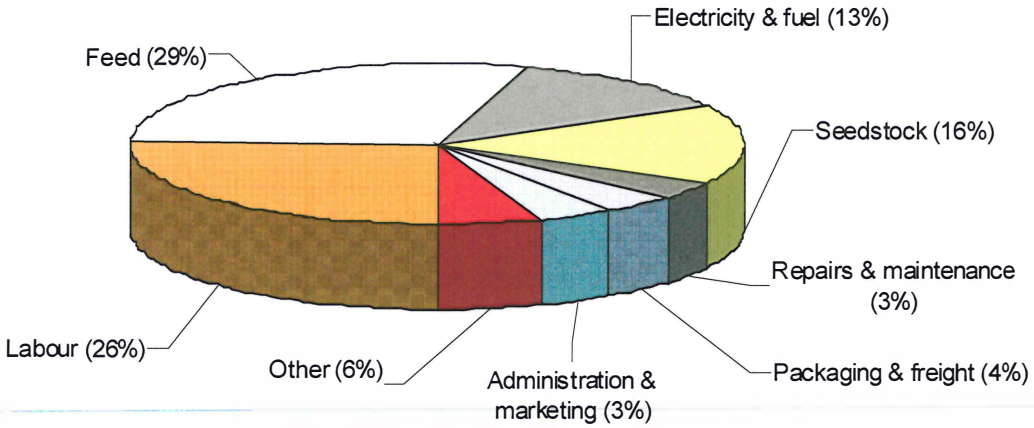

Fig. 8.4. Composition of running costs (mean values) for semi-intensive/intensive Murray cod aquaculture operations

8.6 Farm scale comparisons

The key financial indicators used to compare the profitability of the different scales of production were:

- **Internal Rate of Return (IRR)**

  The Internal Rate of Return (IRR) is the discount rate that equates the present value of net cash flow with the initial outlay. It is the highest rate of interest an investor could afford to pay, without losing money, if all of the funds to finance the investment were borrowed, and the loan was repaid by application of the cash proceeds as they were earned. Conventional projects involve an initial outlay followed by a series of positive cash flows. In this case, if the IRR is higher than the required rate of return then the NPV is positive.

  The initial capital investment that is used to calculate IRR and NPV includes:

  - Capital Goods Purchased
  - Capital Goods and Land Value bought to the venture by farmer
  - Year 1 Running Costs (Working Capital).

- **Profit Margin (PM)**

  Profit Margin (PM) is the sales return before interest. The PM is equal to the Net Income (NI) before interest {NI + after tax interest expense (ATI)} (averaged over 10 years) divided Revenue (averaged over 10 years). This ratio indicates the percentage of sales revenue that ends up as income. It is a useful measure of performance and gives some indication of pricing strategy or competitive intensity.

- **Return on Assets**

  Return on assets is the operating return which indicates the company's ability to make a return on its assets before interest costs. Rate on total assets (ROTA) equals PM times Asset Turnover (AT).

8.6.1 Key profitability indicators

The 1 tonne and 5 tonne farm show a modest net profit after tax which can be used to supplement income of the principal farm activity. However, labour costs are not included in operating costs and therefore the indicators can not be compared to the other 3 larger intensive farms.

Table 8.3 summarises the major financial indicators for each of farms under investigation. The 25, 50 and 100 tonne farms show that they can generate a PM equal to or greater than the current average for the agribusiness sector. PM ranged from 16.1% (25 t farm) to 21.6% (100 t farm). The IRR ranged from 7.1% (1 t farm) to 13.1% (100 t farm). However, there has been recent deterioration of conditions in the agribusiness

sector related to the weaker commodity prices, stronger dollar, lower exports and the effects of the drought. This reveals that the intensive fish farming enterprise may be more economically robust in terms of water use efficiency, domestic demand for fish products and the increasing dependency on farmed fish over the wild catch sector.

Sale price affects both IRR and PM. For all farm sizes, the IRR was zero at sale prices of $7/kg and $11/kg, but was greater than 6.9% at $15/kg and $19/kg (Table 8.4). Positive PM values for all farms sizes were obtained when the sale price was $15/kg and $19/kg, but at $7/kg the PM was negative for all farm sizes, and was negative for the 25 tonne farm at $11/kg (Table 8.4).

Table 8.3. Summary of major financial indicators for Murray cod (@$15/kg) recirculation systems with different scales of production (tonnes per annum).

Description	Integrated systems		Intensive systems		
	1 Tonne	5 Tonne	25 Tonne	50 Tonne	100 Tonne
Cost per kg (inc. Dep.)	$10.69	$10.42	$11.34	$11.07	$10.14
Sales	$17,100	$73,300	$378,000	$778,800	$1,603,000
Net Present Value	$5,774	$43,600	$114,000	$377,800	$1,253,641
Internal Rate of Return (IRR)	7.1%	9.0%	8.0%	10.3%	13.1%
Profit Margin (PM)	19.4%	20.3%	16.1%	17.2%	21.6%
Return on Asset	7.9%	8.8%	11.9%	13.5%	14.7%
Asset turnover	0.4	0.5	0.7	0.8	0.7
Cost Benefit Ratio	1.1	1.4	1.2	1.4	1.6
Net Profit after tax	$3,400	$15,000	$62,000	$135,000	$353,000

Table 8.4. Effect of price on profit margin (PM) and internal rate of return (IRR) on various farm sizes

Description	Integrated systems				Intensive systems					
Price ($/kg)	1 Tonne		5 Tonne		25 Tonne		50 Tonne		100 Tonne	
	IRR (%)	PM (%)	IRR (%)	PM (%)	IRR (%)	PM (%)	IRR (%)	PM (%)	IRR (%)	PM (%)
$7 /kg	0	-34.4	0	-44.2	0	-57.5	0	-53.5	0	-40.3
$11/kg	0	1.7	0	4.7	0	-1.7	0	0.5	0	0.6
$15/kg	7.1	19.4	9	20.3	8.0	6.1	10.25	17.2	13.1	21.6
$19/kg	29.2	24.3	16.1	29	22.5	25.4	21.5	26.7	23.3	30

8.6.2 The impact of risk

During the first few years of production of new farming operations, there is a risk that full or optimal production will not be achieved. Reasons for this may include teething problems with infrastructure and equipment, inexperience in Murray cod farming and husbandry, lack of knowledge and inappropriate management and/or operation strategies. The impact of risk on IRR can be depicted as a learning curve through the first years of production as compared to the optimal level of production for the species and culture system. As an example, the impact of two different levels of risk on production and IRR were evaluated for a newly commissioned 50 tonne Murray cod farm. At optimal production the IRR is 10.3% (Table 8.3). However, at the first risk level (50%, 70% and 85% of optimal production in years 1, 2 and 3 respectively) the IRR is 8%, while at the second risk level (25%, 50% and 75% of optimal production in years 1, 2 and 3 respectively) the IRR is reduced to 5.6% (Fig. 8.5).

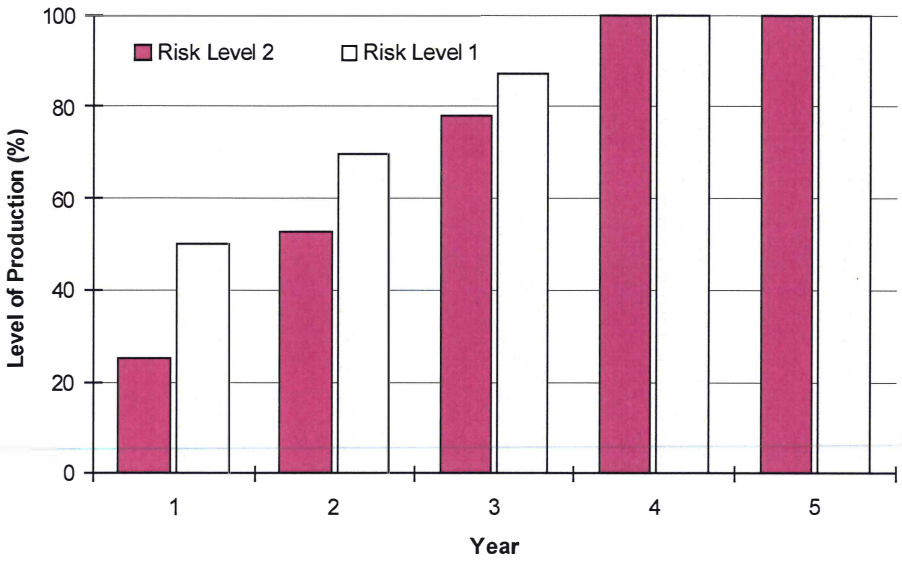

Fig. 8.5. Effect of two different levels risk on production a new 50 tonne Murray cod farm (Risk Level 1 = 50%, 70% and 85% of optimal production in years 1, 2 and 3 respectively. Risk Level 2 = 25%, 50% and 75% of optimal production in years 1, 2 and 3 respectively)

8.6.3 Comparison with other studies

There have been several previous studies which have concentrated on the economic profitability of growing Murray cod in intensive recirculation systems. These were Trapnell (2000), Rawlinson and Forster (2001) and Weston *et al.* (2001). While some of the parameters used in these models differed in terms of price per kg, stocking and harvesting practices, mortality and growth rates, the results give an indicative insight into how the feasibility and associated profitability of Murray cod farming in intensive recirculation systems have changed over time. A summary of the main indicators from these studies and the key parameters that were used in building the farm models are presented in Table 8.5. As the present study does not include labour costs in the 5 tonne farm, for cash flow comparative reasons (to give an indication of labour cost capacity) these indicators should be viewed in terms of exposition rather than comparison.

Trapnell (2000) examined the diversification by graziers into Murray cod aquaculture on a small scale, with the following assumptions. The production level was 1,250kg/annum, and labour was either nil (family labour on the farm assumed to be nil) or $14,820. Trapnell (2000) indicated that intensive Murray cod was labour intensive, and that the cost of family labour was critical in determining levels of profitability and cash flow. When labour was nil the IRR was 25%, but if labour was included in running costs then the IRR was 3%. Profitability in this model requires some running costs to be offset against other farming activities.

The Murray cod farm models constructed by Weston *et al.* (2001) were based on semi-intensive (5 and 10 tonne/annum) and intensive (30 tonne/annum) recirculation systems. These models were used to illustrate sources of risk and uncertainty affecting viability of operations. Weston *et al.* (2001) indicated Murray cod had a high market value and was in demand in some markets, but given that this is a relatively new industry, there was uncertainty as to whether current prices could be sustained if production increased. Results from this study indicated that 5 and 10 tonne farms were not viable but a 30 tonne farm was marginally viable, with a high degree of risk. These results also showed that profitability was sensitive to farm gate price, cost of seedstock and labour costs. Although this study showed that small farms were not viable, integration with other farming practices (agriculture, horticulture, etc) may improve viability.

Table 8.5. Comparison of feasibility and profitability indicators from Trapnell (2000), Rawlinson and Forster (2001) and Weston *et al.* (2001), and the present study.

Farm size (tonne/annum)	Main Indicator / Parameter	Trapnall (2000)	Rawlinson & Forster (2001)	Weston *et al* (2001)	Present Study
1 tonne	Benefit Cost Ratio		-	-	1.1
	IRR	3% & 25%	-	-	7.1%
	Profit Margin (PM)		-	-	19.4%
	Sale Price	$18	-	-	$15
	Sale Weight		-	-	≈500g
	Capital Costs	$50,000	-	-	$35,000
	Feed costs	$1,500	-	-	$1,600
	Labour Costs	0 & $15,000	-	-	n/a
5 tonne	Benefit Cost Ratio	-	-	0.88	1.4
	IRR	-	-	n/a	9%
	Profit Margin (PM)	-	-	n/a	20.3%
	Sale Price	-	-	$15	$15
	Sale Weight	-	-	300-400g	≈500g
	Capital Costs	-	-	$133,000	$150,000
	Feed costs	-	-	$10,700	$15,300
	Labour Costs	-	-	$15,000	n/a
10 tonne	Benefit Cost Ratio	-	-	0.93	-
	IRR	-	-	n/a	-
	Profit Margin (PM)	-	-	n/a	-
	Sale Price	-	-	$15	-
	Sale Weight	-	-	300-400g	-
	Capital Costs	-	-	$209,000	-
	Feed costs	-	-	$21,400	-
	Labour Costs	-	-	$30,000	-
25 & 30 tonne	Benefit Cost Ratio	-	-	1.15	1.2
	IRR	-	16.7%	n/a	8.0%
	Profit Margin	-	23.50%	n/a	16.1%
	Sale Price	-	$20	$17.50	$15
	Sale Weight	-	500g – 1,000g	450-550g	≈500g
	Capital Costs	-	$416,000	$431,000	$380,000
	Feed costs	-		$61,600	$72,000
	Labour Costs	-	$40,000	$40,000	$75,000
50 tonne	Benefit Cost Ratio	-	-	-	1.4
	IRR	-	18%	-	10.3%
	Profit Margin (PM)	-	27%	-	17.2%
	Sale Price	-	$20	-	$15
	Sale Weight	-	500g – 1,000g	-	≈500g
	Capital Costs	-	$916,000	-	$800,000
	Feed costs	-	-	-	148,400
	Labour Costs	-	$80,000	-	$130,000
100 tonne	Benefit Cost Ratio	-	-	-	1.6
	IRR	-	-	-	13.1%
	Profit Margin (PM)	-	-	-	21.6%
	Sale Price	-	-	-	$15
	Sale Weight	-	-	-	≈500g
	Capital Costs	-	-	-	$1,500,000
	Feed costs	-	-	-	$305,600
	Labour Costs	-	-	-	$210,000

Twenty five tonne and 50 tonne farm models were constructed by Rawlinson and Forster (2001). A 150 tonne farm model was also constructed but these data are not included in this comparison. As with the current study, these models used an earlier version of AQUAFARMER™ software to assess feasibility of each farm model. Rawlinson and Forster (2001) showed that both the IRR, return on assets and equity after 10 years were greater for the larger farm. However, in comparison to the present study, there has been a reduction in both IRR and PM for each of the farm models since the study by Rawlinson and Forster (2001). In the case of the 25 tonne farm the IRR has declined from 16.7% to 6.9% and PM has declined from 23.6% to 15.6%. While in the case of the 50 tonne farm the IRR has declined from 18% to 10.3% and PM has declined from 27% to 17.2% (Table 8.5).

8.7 Conclusions

The main conclusions that can be drawn from the present study include:

- Recirculation systems offer greater control of key production and economic variables and afford improved risk management control.
- Key bio-economic variables influencing viability include:
  (a) Scale of the farm
  (b) Species biological attributes (mortality and growth)
  (c) Species market attributes (products and price).
- There are significant opportunities for improved risk management in larger systems.

Victoria, while a small producer of aquaculture products, is leading Australia in its research of Murray cod in terms of fish health, feed developments, product and marketing development. The improvement in investment during the last year reveals a promising future for Murray cod throughout the range of farm scales. Victoria, like the rest of Australia, is searching for ways to improve water utilisation and environmentally friendly systems to produce food products. Recirculation aquaculture provides a manageable solution to farm diversification and stand alone ventures.

Each of the farms analysed reveals very strong indicators of financial success. The PM and the return on assets rival the best performing sectors in the economy. However, it must be remembered that the data is dependent on best practice husbandry and recirculation technology. The farm models present a best case scenario that assumes optimal production (100% production throughout a ten year project) and sale of all output once fish have completed their grow out period. This may not be the case in reality, as real time data will change from year to year. However, the model farms do give an indication of the inherent viability of growing Murray cod in recirculation aquaculture systems.

Previous studies on the economics of RAS have indicated that economies of scale can be achieved (Wade *et al.* 1996; Rawlinson and Forster 2001; Weston *et al.* 2001). In the present study, the influence of production scale on the cost of production (per kg) reveals a 4% fall in that cost from a 25 tonne farm to a 50 tonne farm and a 10% fall from a 50 tonne farm to a 100 tonne farm. Overall, the reduction in the cost of production from moving from a 25 tonne farm to a 100 tonne farm is in the order of 12%. The PM, on the other hand, shows an increase of around 40% when moving from a 25 tonne farm to a 100 tonne farm.

Figures presented in the present study are strongly influenced by the configuration of annual running costs, for example feed and labour accounts for between 45% - 60% of total costs. Further declines in price of farmed Murray cod may be offset by improvements to productivity. Areas that may improve profitability of production include:

- Use of labour saving devices such as automatic feeding systems and mechanical grading systems.
- Adoption of more efficient recirculation aquaculture technology to reduce running costs (eg. Pumping and heating systems).
- Improved husbandry techniques to optimise growth and survival, which will reduce cost of fingerlings and time to attain market size.
- Use of a more cost effective diet (cheaper ingredients and lower FCR's). A more cost-effective Murray cod diet has been specifically developed for Murray cod which has a lower purchase price and better FCR than diets currently used (see De Silva *et al.* 2004), but this diet has yet to be taken up by industry.
- Use of genetically improved seedstock with enhanced growth, survival and food conversion efficiencies.

- For smaller operations, offsetting running costs by on-farm diversification, such as offered by integrated agri-aquaculture systems (Gooley and Gavine 2003).

8.8 Acknowledgments

This article was compiled with data obtained from commercial Murray cod aquaculture operations collected during 2002. The author wishes to thank the operators of these farms, that wish to remain anonymous, for their assistance and Brett Ingram (PIRVic, Snobs Creek) for providing information on growth and production of Murray cod.

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9 OUTCOMES OF THE FRDC-FUNDED MURRAY COD AQUACULTURE PROJECT (1999/328)

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CONTENTS

9.1 Introduction

The Murray cod is highly valued and sought after as a table fish. Until the development of grow-out techniques for Murray cod, markets relied on a small, declining, wild commercial fishery that provided limited quantities of fish of highly variable quality on a seasonal basis. However, with the development of captive spawning techniques in the early 1980's, a hatchery-based industry for fingerling production is now well established in Victoria, NSW and Queensland, and in excess of one million fingerlings are produced annually. Since the early 1990's, methods for the commercial production market-size Murray cod continue to be developed and refined, much of which is described in the present report (Ingram and De Silva 2004). Currently, Murray cod aquaculture is seen as a new and expanding industry both within Australia and overseas. Market size Murray cod are now routinely cultured in aquaculture facilities in NSW, Queensland, Victoria, South Australia and Western Australia.

Prior to commencing the current project, specific industry needs for Murray cod R&D were identified through industry and government consultation (Ingram *et al.* 2004). Briefly, the key needs were categorised into four priority areas:

- Fish health (minimising stress and maximising survival and growth)
- Genetic improvement (selection for improved performance while protecting wild genetic stocks)
- Diet development (feeds and feeding regimes)
- Market information and bio-economic modelling.

The present project was undertaken to address some of these R&D priorities and to facilitate development of the Murray cod aquaculture industry.

9.2 Conclusions from R&D

9.2.1 Industry status

Murray cod aquaculture is a small but growing industry within Australia. Murray cod responds well to commercial production in recirculating aquaculture systems (RAS) where most production is occurring. In August 2002, approximately 60 farms had Murray cod on site, and 19 farms had sold marketable fish (table-size fish). The level of production has ranged from < 1 t/annum/farm to over 100 t/annum/farm. Most production is currently confined to Victoria, NSW and SA, but farms have been established in Queensland and Western Australia, as well as in some southeast Asian countries. The level of production of market-sized Murray cod has increased substantially over the past few years, and in 2000/01 over 100 tonne was produced in Australia. The total value of the Murray cod aquaculture industry (hatchery production of fingerlings and table fish production combined) was $2.48 million/annum in 2001/2002, with the grow-out sector representing 80% of the total industry value.

9.2.2 Fingerling production and growout

The present study has considerably broadened the knowledge on the culture of Murray cod particularly the weaning of post-larvae and fingerlings, and grow-out in tanks and cages under controlled and ambient conditions, respectively. These preliminary findings showed that Murray cod perform well especially under intensive culture conditions, as indicated by excellent growth rates, survival rates and FCR's at high stocking densities. Initial acclimation of new stock to aquaculture conditions, and initial stages of feeding (weaning) were identified as critical phases in the successful culture of Murray cod. However, the present study showed that both post-larvae and fingerlings could be successfully weaned onto an artificial diet.

The data collected have provided key production information for the grow-out of Murray cod to a minimum market-size (700 g), including growth, survival, fish condition, feeding rates, and food conversion ratios, related to both fish size and culture temperature. Based on these data, for every 1,000 fingerlings (1g/fish) stocked into a commercial RAS, operating at temperatures between 22-26°C, between 413 and 616 kg (median 500 kg) of Murray cod (at 700 g/fish) are expected to be harvested from 36 weeks to 108 weeks after stocking. Information collected during cage culture trials suggested that Murray cod, at least stocked as "young-of-year", could be successfully reared in cages under ambient conditions. These results have been used to analyse the economic viability of Murray cod farming systems (see Section 9.2.7).

9.2.3 Nutrition

Murray cod is a top order carnivore, and as expected, requires a dietary protein content of about 50%, by dry weight, for optimal growth performance. Nutritional studies found that Murray cod is able to digest food industry by-products, such as defatted soybean meal, shark meat meal and meat meal, effectively. The "protein sparing" abilities of Murray cod are relatively limited. However, up to 32% of the fish meal content in Murray cod diets could be replaced without compromising growth performance, food conversion efficiency, protein efficiency ratio and net protein utilisation. Further, since the fatty acid content of Murray cod muscle reflects that of the diet, feeds could be formulated in such a manner as to satisfy consumer acceptability or to increase the n-3 levels with out compromising growth performance. Sufficient information was provided to develop a relatively cost-effective commercial diet for the intensive culture of Murray cod, which was successfully tested under commercial conditions. Adoption of this diet for grow-out of Murray cod may significantly reduce the overall cost of production.

9.2.4 Water quality

There have been no previous attempts to determine the optimal water quality requirements for Murray cod, either in the wild or under aquaculture conditions. The present study investigated water quality in both commercial and experimental culture systems for Murray cod and provided valuable baseline information on water quality conditions in which Murray cod have been cultured. Values for 16 water quality parameters were summarised to provide a guide to what may be considered acceptable ranges for commercial intensive Murray cod aquaculture.

A simplified nutrient mass balance model was developed to estimate the amounts of nitrogen (N) and phosphorus (P) discharged from an intensive Murray cod aquaculture system. Based on median nutrient input values, for every tonne of market size Murray cod harvested approximately 12.4 kg P/tonne and 67 kg N/tonne are produced. Changes in FCR and the amount of N and P in the feed strongly influenced these values.

Water quality within RAS is complex and dynamic, and influenced by the various water treatment components within the system, interactions with different water quality parameters and various farming operations. Management of the water quality is critical to the overall performance of RAS. Results from the present study have implications for the future construction, operation and management of RAS systems for the culture of Murray cod.

9.2.5 Fish health

An industry survey indicated that the most commonly encountered health problems in Murray cod aquaculture were: protozoan parasite infestations (28% of respondents); water quality problems (28%); fungal infections (19%); non-specific bacterial infections (17%); nutritional problems (11%); and predation/cannibalism (11%). The known fish diseases and fish health issues affecting Murray cod were described, including bacterial, protozoan fungal and metazoan parasites, nutritional diseases and diseases associated with water quality. Strategies to assist farmers in the management of the health and well being of stock in intensive aquaculture facilities were discussed. These included water supply sources and options for its treatment, quarantine and management of new stock, management of hygiene in aquaculture facilities, monitoring stock and identifying health problems and use of drugs and chemicals. A decision support pathway was presented to assist farmers in the identification and management of major health problems in Murray cod aquaculture operations.

9.2.6 Markets and marketing

Currently, a variety of Murray cod products are now routinely cultured for use in a range of markets from post-larvae and fingerlings for stock enhancement and grow-out, to table fish (0.6 – 4.0 kg) sold live, fresh, gilled and gutted, and as fillets into a number of markets. Initially farmed Murray cod supplemented the existing wild capture fishery, only to replace it in the markets following the closure of that fishery. Farmed Murray cod have expanded into new markets such as the live fish market, and there has been improved market chain efficiency through more reliable and routine supply of a product of known quality. Prices paid for farmed Murray cod over the past three years have declined from a high of approximately $24.00/kg in late 1999 to a steady price of around $13.50/kg.

Information was provided on the marketing of Murray cod from an industry standpoint. The Australian inland aquaculture industry, which includes Murray cod, requires the development of an industry wide marketing strategy. Within Victoria, an industry driven Murray cod marketing co-operative was proposed to facilitate the development of this species. The co-operative will need to appraise suitable market opportunities and establish appropriate economic and quality assurance benchmarks, while ensuring access to a critical mass of production on a regular basis. Future opportunities for growth of the Murray cod aquaculture industry may be driven more by market demands and perceptions such as supply of environmental friendly or organic products improved product processing and presentation.

9.2.7 Economic analyses

The financial and economic efficiencies were investigated for five RAS producing 1, 5, 25, 50 or 100 tonne/annum Murray cod. Production data (growth, FCR, mortality, equipment and running costs) from industry were combined with results from the present study and analysed using AQUAFARMER™ feasibility software to determine and evaluate the bio-economic performance of each system in terms of key financial indicators, internal rate of return (IRR) and profit margin (PM).

The composition of running costs (mean values) for a Murray cod operation were presented. The highest running cost was labour (27%), followed by feed (26%) and seedstock (16%). However, running cost variables changed with increasing farm size. Each of the farms analysed revealed strong indicators of the inherent financial viability of growing Murray cod in RAS, though configuration of annual running costs and sale price were strongly affected by both IRR and PM. The study showed that on-farm diversification

would improve viability of smaller farms while there were significant opportunities for improved risk management in larger systems and that economies of scale can be achieved. This study indicated that options to improve profitability of production included adoption of more energy and labour efficient recirculation aquaculture technology, improved husbandry techniques to optimise growth and survival, use of a more cost effective diet and use of genetically improved seedstock with enhanced growth, survival and food conversion efficiencies.

9.3 Future directions

9.3.1 R&D Needs

9.3.1.1 Production systems

Murray cod is one of the primary species under culture in freshwater RAS, along with other species, in both polyculture and monoculture situations, including eels (*Anguilla* spp.), barramundi (*Lates calcarifer*), jade perch (*Scortum barcoo*), sleepy cod (*Oxyeleotris lineolatus*) silver perch (*Bidyanus bidyanus*) and salmonids (O'Sullivan 1999; Larkin 2000; O'Sullivan 2000/2001; Mosig 2003/04). There is a strong emphasis on the use of innovative aquaculture technology in RAS. The advantages of RAS include compactness (small footprint), biosecurity, high density production and their ability to control the environment in which the animals are reared. RAS are generally more environmentally friendly than other types of aquaculture systems due primarily to their reduced water usage and waste discharge. Not surprising, RAS are attracting immense interest in Australia and the number of systems in operation continues to grow substantially. However, cost of production in RAS, compared to pond-based systems, is high and reductions in farm gate prices can strongly impact on their viability.

Further development of the Murray cod aquaculture industry should now focus on improving the efficiency and reliability of the culture systems themselves to enhance production. Additionally, with increasing emphasis on environmentally sustainable development of food production methods, there is a need to support and promote use of RAS technology across the aquaculture industry. There is a need to provide R&D to support the sustainable development of RAS-based industries. Information on system components, design and performance characteristics and standards is required to reduce the risk of system failure and improve production certainty and efficiency. Although, due to the nature of RAS, small volumes of water are discharged relative to the level of production, cost effective and environmentally sound options for treatment and disposal of this waste stream need to be explored. These may include improvements to the water treatment process such as incorporation of phosphorus stripping and denitrification technology, and utilising the discharged water for irrigation purposes.

Some of these issues are now being addressed. In Victoria, the Vic-RAS-Net (Department of Primary Industries) provides a support network for the RAS industry through provision of an environment in which information is shared directly between members through *ad hoc* field days, workshops and newsletters. Deakin University has recently obtained funding from the Victorian Government Science Technology and Innovation Initiative (STI) to undertake industry scale research into the use of RAS (Anon 2003). The Department of Primary Industries, with funding from Fisheries Victoria, is currently preparing Best Practice Environmental Management Guidelines for the RAS industry in Victoria (O'Sullivan 2003). Various state fisheries and aquaculture agencies have undertaken industry-based workshops and some teaching institutions are now providing training in use of RAS.

Earthen ponds are widely used for the culture of freshwater fish worldwide. In fact, more finfish are produced in open ponds than any other culture system (Landau 1992). Integrated agri-aquaculture systems (IAAS) link aquaculture to existing, conventional agricultural farming systems. Development of these systems across rural Australia is being driven by initiatives to enhance farm productivity and water use efficiency (Gooley 2000; Gooley *et al.* 2001; Gooley and Gavine 2003). IAAS typically utilise both earthen ponds and floating cages within agricultural water-bodies for aquaculture. Although most production of Murray cod is currently occurring in RAS, results from the present study suggested that seasonal (southern states) or even year-round culture (northern climates) in cages and ponds under ambient conditions may be possible. Use of ponds to "finish-off" fish prior to market is already occurring on some farms. However, there is a need to further assess the performance of Murray cod in cages and ponds under different conditions over extended periods. As growth of Murray cod is affected by temperature, there is also a need to investigate options for increasing and maintaining temperatures in outdoor culture systems (eg. use of

greenhouses), especially in southern states.

Production systems R&D needs
• Improve efficiency and reliability of RAS.
• Develop and assess cage and pond culture techniques in association with IAAS

9.3.1.2 Broodfish and seedstock production

The status of Murray cod was recently reviewed (Kearney and Kildea 2001) and due to declines in abundance and distribution in the wild and threats to habitats inhabited by Murray cod, in July 2003 the species was listed as a vulnerable under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) (http://www.deh.gov.au/epbc/index.html). A vulnerable species is one that is facing a high risk of extinction in the wild in the medium-term future, as determined in accordance with prescribed criteria outlined in Section 179 of the Act (see http://www.deh.gov.au/biodiversity/threatened/species/index.html). As a result, it is anticipated that regulations protecting Murray cod in the wild will be reviewed and tightened by respective state authorities. Commercial fishing for Murray cod is now virtually banned in all states, and the listing of the species under the EPBC Act will affect export of the species. Access to broodfish from the wild is also likely to become severely restricted.

Broodfish collected from the wild are required for stock enhancement programs to ensure maintenance of the genetic integrity and diversity of wild populations. Preliminary research has suggested that Murray cod fingerlings being stocked from hatcheries may not be representative of the wild populations in terms of the number and proportions of haplotypes (Bearlin and Tikel 2003). Therefore, there is a critical need to review existing hatchery practices, particularly broodfish procurement, and managing breeding programs to minimise loss of population genetic diversity by genetic drift where the effective hatchery population size is small (Ryman 1991; Yokota *et al.* 2003).

Conversely, for the Murray cod grow-out industry to continue to expand there is a need to eliminate reliance on wild fish as broodfish. Some Murray cod hatcheries are increasingly using self-propagated progeny as new broodfish, often with indifference to the genetic implications. Significant problems arise in poorly managed breeding programs, which can severely effect the long-term sustainability and economic viability of aquaculture industries (Tave 1993). There is a critical need to develop technologies and guidelines for broodfish management and selective breeding, and associated risk analysis, to ensure the long term genetic integrity and improvement of farmed Murray cod stocks for grow-out purposes. (A more detailed discussion of genetics R&D needs for Murray cod is *Genetics and biotechnology* section below)

Production of Murray cod seedstock is an established industry with in excess of one million fingerlings produced annually. Between 25% and 50% of Murray cod fingerlings produced annually are used in grow-out industry. However, supply and availability of fingerlings is still highly variable, being affected by the seasonal nature of spawning in Murray cod, seasonal variation in production levels related to size/maturity, number and condition of broodfish, as well as changes in environmental conditions that may affect spawning. There is a continuing and increasing demand for Murray cod seedstock for both stocking and grow-out domestically, as well as for export to overseas markets. For the hatchery industry to meet this growing demand there is a need to increase production.

Increasing broodfish numbers, and ensuring the healthy condition of these fish will increase production levels. This will be further facilitated by being able to access broodfish that have been reared completely in captivity (see *Broodfish* section) and selected for enhanced seedstock production (see *Genetics and biotechnology* section). Most hatcheries currently rely on the natural spawning of broodfish in ponds for the production of seedstock. Although this method is relatively cost effective, there is no control of the mating events, which individuals are spawning together and when. There is a need to move towards more controlled methods of spawning to support selective breeding programs. Increasing the availability of seedstock, especially for the grow-out industry, may be achieved by developing "out-of-season" spawning

of Murray cod using broodfish held in environment control systems. Preliminary research indicates this is possible, but levels of fingerling production and associated bio-economic benefits that can be achieved by this method have yet to be determined. Similarly, bio-economic analyses will need to be undertaken to determine the effect of a mid-season intake of seedstock, as opposed to a single intake of seedstock during the normal season (December-March), on production in intensive grow-out facilities.

Broodfish and seedstock production R&D needs
• Develop guidelines for procurement of wild fish and management of broodfish and breeding programs for production of seedstock used in stock enhancement programs. • Develop management guidelines for closed breeding systems in which self-propagated broodfish are used for production of seedstock for the grow-out industry, taking into account genetic requirements of selected breeding programs. • Increase production and availability of seedstock. • Develop and refine controlled spawning techniques, including 'out-of season" spawning.

9.3.1.3 Genetics and biotechnology

Use of biotechnology in the aquaculture industry is growing rapidly, as indicated by increasing R&D activity and investment in this field. Indeed a vast range of possible applications of aquatic genetic resources is emerging. Well-managed breeding programs incorporating selection of favourable traits (growth, survival, disease resistance, food conversion efficiency etc.), have resulted in tremendous gains in production performance and reliability, and increased profitability of culture animals (Tave 1993; Gjedrem 1997). For example, selection in channel catfish (*Ictalurus punctatus*) has resulted in 12-18% gain in growth rate after one generation (Dunham 1987). In coho salmon (*Oncorhynchus kisutch*), 10 generations of selection improved growth rates by 50% (Hershberger *et al.* 1990), while selective breeding of brown trout (*Salmo salar*) and brook trout (*Salvelinus fontinalis*) has lead to strains that are resistant to the bacterium *Aeromonas salmonicida* (Ehlinger 1977; Cipriano *et al.* 2002). The Murray cod aquaculture industry is in its infancy and consequently the potential to increase profitability of production through instigation of an industrial scale selective breeding program is considerable (Hayes 2002).

The fact that some Murray cod farmers are now using fish reared totally in captivity as broodfish indicates that, intentionally or inadvertently, selective breeding programs are being instigated on a farm specific level, and often without the necessary skill and technical information or indifference to the genetic implications. Significant problems arise in poorly managed breeding programs, which can severely effect the long-term sustainability and economic viability of aquaculture industries. For example inbreeding and loss of genetic variation results in increased abnormalities, low survival low disease resistance and slow growth, which can severely affect the long-term sustainability and economic viability of aquaculture industries (Tave 1993). Indeed, these concerns have already manifested themselves in the relatively young silver perch aquaculture industry. The incidence of abnormalities and deformities in silver perch seedstock has increased and the overall performance in grow-out facilities has declined (Dr. Sturt Rowland NSW Fisheries, *pers comm*). There is a critical need to develop technologies and guidelines for selective breeding and associated risk analysis to ensure the long term genetic integrity and improvement in performance of farmed Murray cod stocks.

Several other biotechnology options are available to enhance production and associated performance of fish in selective breeding programs. These include chromosome manipulation (polyploidy, gynogenesis and androgenesis), sex manipulation and hybridisation (eg. Dunham 1990; Tave 1993; Pandian and Koteeswaran 1998; Arai 2001; Lutz 2001). None of these applications have been applied to the aquaculture of Murray cod. It is noted, however, that hybridisation between Murray cod and trout cod (*M. macquariensis*) has already been reported in the wild and has been induced in the hatchery (Douglas *et al.* 1995). Some of these

applications also provide a level of biosecurity through sterilisation of progeny, reducing the risk of contamination of wild populations from escapees from aquaculture facilities, and protecting investment in genetic breeding programs from unwanted and indiscriminate exploitation (Dunham 1990; Pandian and Koteeswaran 1998). Selective breeding programs can also be enhanced through access to gene banks of cryogenically preserved gametes (Chao and Liao 2001). Cryopreservation has many practical applications in breeding programs. In particular, sperm form a wide range of genetically diverse stock, or from fish with specific genetic traits favourable for aquaculture, may be stored and managed cost effectively, and be later utilised in either selective breeding or conservation programs. To date, milt from other species of *Maccullochella* have been cryopreserved (I. Gunn, Monash Institute of Reproduction and Development, Clayton, *pers com.*), but no attempts have been made to assess the viability of this material for breeding following thawing.

Genetics and biotechnology R&D needs
• Establish a selective breeding program to ensure the long term genetic integrity and improvement of farmed Murray cod stocks.
• Apply chromosome manipulation, sex manipulation and hybridisation techniques to Murray cod aquaculture and assess performance of progeny and associated value to selective breeding programs.
• Establish a cryopreserved sperm bank for use in selective breeding and biodiversity conservation programs.

9.3.1.4 Nutrition

Protein is the single most expensive ingredient in fish feeds and fish meal is the major dietary protein source of fish feeds. With the rapid expansion of the aquaculture industry and the increasing demand for seafood products by consumers, the demand for fish meal and fish oil in aquaculture feeds is predicted to triple over the next decade (Watanabe 2002). The vast majority of fish meal and fish oil is derived from wild fisheries, a number of which are fully exploited, over-exploited or depleted. Consequently raw ingredients are becoming scarcer or more costly. For the aquaculture industry to grow in a sustainable manner it must reduce fish meal input from wild fish and adopt ecologically sound practices (Naylor *et al.* 2000). Consequently, fish meal and fish oil replacement in aquaculture feeds has become a challenge for finfish nutritionists, feed manufacturers and fish culturists. Reducing dietary protein through protein sparing is limited in Murray cod, yet the present study showed that up to 32% of the fish meal content in diets could be replaced without compromising performance.

The present study found that feed represented a significant (mean 26%) running cost in intensive Murray cod aquaculture facilities. As previously indicated, there is a need to improve the overall efficiency and reliability of RAS. This may be achieved by reducing manufacturing cost of feed (cheaper ingredients etc.) and improving feed management strategies for optimising fish growth and minimising wastage. Although the present study provided baseline information to develop relatively cost-effective commercial diets for Murray cod, and a diet was produced that performed better under commercial conditions than diets currently used by industry (salmonid and barramundi diets), no commercial feed manufacturer has taken up production of this diet for industry. This is unlikely to occur until the level of production of Murray cod has increased substantially. In the present study, some feeding trials showed that feed wastage was as high as 17.6% per day. Feed wastage must be minimised in RAS as uneaten food can increase nutrient levels within the system, placing added and unnecessary loading on both mechanical and biological filtration systems. Feeds that are more water stable and retard leaching of nutrients can be better managed in RAS. The risk of environmental impact associated with discharge of effluent water containing high levels of nutrients, particularly nitrogen (N) and phosphorous (P), is a problem for some aquaculture industries and operations. Undigested, unutilised and wasted feed is the main source of these nutrient wastes. Further development of diets should include investigations to reduce P and N content without compromising performance. Critical to RAS performance and viability are food conversion efficiencies, which can be optimised by combining the

use of nutritionally balanced feeds with Best Practice feeding management strategies. Adoption of the use of demand and automatic ("smart") feeding systems (eg. Zeigler and Johnson 1998; Tsukuda *et al.* 2000) will improve feeding efficiency.

Since the fatty acid content of Murray cod muscle reflects that of the diet, feeds could be formulated in such a manner as to satisfy consumer acceptability or to increase the n-3 levels with out compromising growth performance. Indeed, this allows for the option of manufacturing more cost effective diets for grow-out, then switching to a "finishing-off" diet just prior to harvest to modify fatty acid content to suit the market.

Nutrition R&D needs
• Explore options for reducing both feed costs and dependency on fish meal and fish oil, through use of alternative protein and oil rich ingredients.
• Develop feed management strategies for intensive aquaculture systems.
• Develop environmentally cleaner diets that have reduced P and N wastes.

9.3.1.5 Fish Health and water quality

Diseases that affect Murray cod in aquaculture have been described, but as the industry grows, new and potentially threatening diseases may arise. For example, a new iridovirus-like virus has recently been recorded from juvenile Murray cod held in a recirculating aquaculture system (Lancaster *et al.* 2003). This infection resulted in 90% mortality in fingerlings (40-60 mm) and 25% mortality in larger fish (100-150 mm). The potential impact of this virus across the industry, including stock enhancement programs, has yet to be assessed. There is a need to promote the use of effective on-farm fish health management strategies to ensure the biosecurity of culture systems and the maintenance of health and well-being of stock. Key actions, which are critical to achieving this, have been described in this report. In particular, industry should ensure that all new stock are quarantined and certified disease free to prevent inadvertent introduction of unwanted pathogens into culture systems.

The Murray cod aquaculture industry needs to adopt and promote a "clean" and "green" product approach. The industry should only use chemicals and drugs that are either registered for use by the Australian Pesticides and Veterinary Medicines Authority (APVMA) (http://www.apvma.gov.au), covered by a APVMA Minor Use Permit or are exempt from registration. Currently, however, today very few chemicals have been registered for use in finfish culture in Australia. There is a need to continue to explore environment friendly therapeutic options for use in RAS that can be registered or covered by a Minor Use Permit. Use of genetics has already been identified as a possible method of improving disease resistance in Murray cod (see *Genetics and biotechnology* Section above). Another option may be the use of probiotics (microalgae, yeasts, and bacteria), which are micro-organisms or their products that have health benefits to the host (Gatesoupe 1999; Irianto and Austin 2002). There is accumulating evidence that probiotics are effective at inhibiting a wide range of fish pathogens and some probiotics have already been used in aquaculture as a means of disease control, supplementing or even in some cases replacing the use of antimicrobial compounds (Irianto and Austin 2002). Probiotics may competitively exclude or actively inhabit the colonisation of less favourable micro-organisms and potential pathogens, and/or stimulate host immunity. Probiotics may also stimulate appetite, and improve nutrition, health and growth (Irianto and Austin 2002). Vaccination has proven to be successful against important bacterial diseases in salmonids, such as enteric redmouth disease. While, there are currently no serious bacterial diseases in Murray cod, use of vaccines by the industry may be required in the future.

This study provided valuable baseline information on water quality conditions in which Murray cod have being cultured. However, water quality requirements for Murray cod aquaculture are still not well defined and these results do not provide an indication as to whether or not concentrations of the parameters measured affected growth of fish. Nevertheless, managing water quality is critical in optimising Murray cod production in RAS and consequently there is a critical need to determine optimal ranges and critical levels of key water quality parameters that influence fish health and growth at all stages of production. Key

parameters include pH, dissolved oxygen, dissolved carbon dioxide, ammonia and nitrite. There is a high degree of inter-relationship between these as well as other water quality parameters within RAS, which means that a variation in one parameter can influence the toxicity of others. Further, the design, operation and management of RAS can profoundly affect water quality. More detailed investigations into the effects of RAS design, operation and management on water quality dynamics are required.

Fish health and water quality R&D needs
• Develop effective on-farm biosecurity programs and fish health management strategies. • Evaluate new, alternative and safe methods of controlling diseases in farmed Murray cod, including the use of probiotics. • Determine optimal ranges and critical levels of water quality parameters that affect fish health and growth.

9.3.1.6 Marketing and Bio-economics

The Murray cod aquaculture industry itself needs to be market-driven to ensure sustained viability, competitiveness and growth. The value of marketing and promotion through both generic and specialised programs cannot be understated and must be an integral part of the business. Continual surveying of critical components of the supply chain is required to ensure that consumer demands are being met. There is a need to explore strengths, weaknesses, opportunities and threats of present food chains and how they can be enhanced in both domestic and international markets.

The industry must supply consistent quantities of safe, high quality products to have a competitive advantage over competing products. One challenge for this industry is to substantiate claims of quality and safety. Codes of practices for quality assurance as well as the principles of Hazard Analysis Critical Control Point (HACCP) need to be implemented to ensure consistent product quality. Such an approach is necessary to maintain a "green" and "clean" image of the products produced and should be a key component of the marketing strategy.

Some farmed Murray cod have been downgraded at markets because of the presence of muddy/earthy taints (flavours) in the flesh. Incidence of these cases reflects poorly on the product and the industry as a whole and therefore should be avoided. Purging of fish is the generally accepted method for removal of earthy taints, however the techniques for adequate purging of Murray cod are yet to be determined.

The role of value adding is to expand the industry and increase profitability from the producer to the retailer. Value-adding options must cater to customer (wholesaler, retailer and consumer) requirements and give the perception of more value without reducing profit margins to producers and processors. Value-adding factors include shelf life (eg. use of modified atmosphere packaging), appearance (presentation), health value, taste, freshness, product range, consumer convenience (easier to prepare) and consistency and reliability of supply. Use of recognisable product branding may also improve market profile. With the increasing use of self-service in large retail outlets, away from counter-service, sale-ready products requiring minimal in-store handling and re-packing, and that are easier to display and stack on shelves, may be more appealing to these retailers. Value-adding factors are also likely to vary for different customers and markets.

It is imperative that existing bio-economic models for Murray cod aquaculture are maintained, refined and updated to undertake on-going monitoring of the economic profitability of production systems, and to support meeting ESD objectives (see FRDC & DNRE 2002) required for the long-term viability of the industry. This will require the continued updating of running costs and fish performance data. These models are not only important to existing farmers to undertake on-going assessment of their operations, but also assist business and investment planning for potential new entrants into the industry. These models must be dynamic, interactive and provide sufficient information to attract investment from the corporate sector (for intensive large-scale and relatively capital expensive RAS) and lending institutions (for

owner/operators etc.).

There is an on-going need to investigate options for reducing running costs. Some have already been discussed in previous sections (ie see *Production systems*, and *Nutrition* sections). In RAS, to realise optimal (ie maximum) production of a facility requires maintaining the daily feed allotment at a maximum level through the year (Hankins *et al.* 1995). However, this is difficult to achieve and not practical, as it requires continual harvesting (daily) of marketable fish and their replacement with new stock. Under current practices the intake of Murray cod seedstock is seasonal (December-March) and year-round production of marketable fish is achieved by the inherent variation in growth rates of individual fish and controlling growth (feed and/or temperature manipulation). One option to partially meet optimal production in RAS-based culture facilities is to employ a sequential production strategy in which harvestable fish are removed and a new cohort of seedstock is added at regular time intervals (Westers and Weeks 2003). Increasing the availability of seedstock may be achieved by developing "out-of-season" spawning of Murray cod (see *Broodfish and seedstock production* section). Bio-economic analyses will need to be undertaken to determine the effect of a mid-season intake of seedstock, on production levels and overall farm performance.

Marketing and bio-economics R&D needs
• Develop appropriate marketing and promotion strategies.
• Monitor markets and develop value-added products that meet market demands/expectations.
• Develop codes of practices and HACCP programs that ensure product quality.
• Maintain, refine and update bio-economic models for Murray cod aquaculture.

9.3.2 Industry development

A vertically integrated Murray cod aquaculture industry is envisaged for Australia, with some elements, already in place (Fig. 9.1). Key components include three phases of Production, Hatchery, Nursery, Grow-out, and a four-tier market component. The various growth stages are:

- *Post-larvae*: seedstock for nursery and/or growout operations.
- *Fingerlings*: recreational and conservation stock enhancement and seedstock for growout operations.
- *"Young of year"* and *sub-adults*: recreational and conservation stock enhancement, and for sale to growout operations (ie. pond/cage culture). Small numbers may also be selected for genetically improved/domesticated broodfish.
- *Table fish*: human consumption. Small numbers may also be selected for genetically improved/domesticated broodfish.
- *Broodfish:* initially sourced from wild stocks (especially to support stock enhancement) but increasingly being sourced from within the industry.

Some operations may encompass all production phases, hatchery, nursery and grow-out, supporting both their own needs as well as supplying other industry needs.

The industry is expected to expand as new operators enter the industry and production methods and operations are improved. At the current rate of growth, the level of production is expected to exceed 500 tonne/annum by 2005/06. Planning for the growth and marketing of the increasing supply of aquaculture products, including Murray cod, for both domestic and export consumption is seen as essential at a national, state and regional level. Industry development plans may be encompassed in a broad national aquaculture strategy relevant to all producers, which might then ultimately filter down to a more detailed plans for specific industry sectors, species, regions and even farms. The more established and experienced producers of Murray cod will need to be the key drivers of this planning process as such a plan will require their experienced input and must be applicable to them if it is to be implemented.

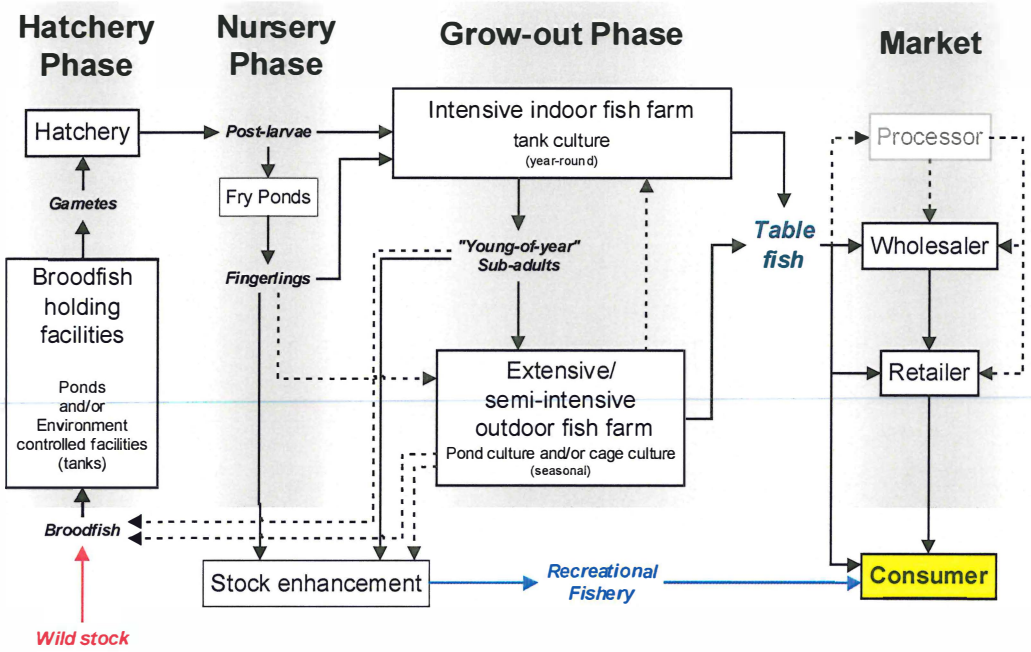

Fig. 9.1. Conceptual Murray cod aquaculture industry model. Solid lines indicate current activities. Dashed lines indicate potential activities through further industry development and expansion.

ESD, which attempts to balance the environmental, economic and social aspects of fisheries and aquaculture, is becoming a major objective of fisheries legislation. A key component is the need to measure and report performance against ESD objectives (FRDC & DNRE 2002). Industry needs to embrace ESD principles and promote best management practices and quality assurance programs across all phases of production.

Individual owner-operator farms are common across the Murray cod industry. While, this type of industry model is attractive, providing regional/rural socio-economic values, this approach may be inconsistent if the goal is a modern, industrial-scale market-driven industry.

Large-scale production to meet demands of large retail chains requires:

- reliable supply of large volumes year-round.
- flexibility and control of whole-chain production and distribution.
- market comparativeness through economies of scale.
- ability to update equipment and apply new technology rapidly.
- Committed mass marketing and promotion strategy.

Small-scale operations find it difficult to meet these conditions, for various reasons including technical, and financial limitations. For the Murray cod industry to make substantial increases in production and market access, both domestically and internationally, requires corporate investment in large-scale production. Production using RAS is a capital-intensive endeavour and for the most part requires corporate investment. Nevertheless, there is still a place for owner-operator farms, particularly in rural areas, which are in a position to supply local and/or specialised niche markets. Integration of existing production systems with agricultural practices, "integrated agri-aquaculture systems" (IAAS) (Gooley *et al.* 2001; Gooley and Gavine 2003), will provide options and opportunities for improving production efficiencies, achieving economies of scale and increasing market access.

9.4 Conclusions

The Murray cod aquaculture industry has been established itself as a relatively small but viable industry that produces a relatively high-valued product that is in demand. Farmed Murray cod have replaced wild caught Murray cod in the market. Results from the present study have provided significant baseline information pertinent to all aspects of Murray cod aquaculture, from fingerling production and grow-out to marketing and sales. To date, development and support of the Murray cod aquaculture industry has been undertaken through state and federal government investment in R&D, as recognised in the current project, which was jointly funded by Fisheries Victoria and the Fisheries Research and Development Corporation (FRDC) and academic institutions, such as Deakin University, Warrnambool. Some on-farm R&D has been undertaken by individual farmers, both independently and in collaboration with research and academic institutions. Support from aquaculture associations has by and large been limited, reflecting the small size of the Murray cod aquaculture industry.

Some academic institutions (university and TAFE) are providing education and training in aquaculture in general. However, there is a need to ensure that these services also incorporate specific training needs of the Murray cod aquaculture industry. Appropriately trained technicians and operators are required for on-going improvements to production and ensuring production performance and efficiency.

Murray cod aquaculture is still a fledgling industry and will continue to need R&D funding support from government to foster growth in the short to medium term. It is recognised that R&D support from within the industry and related industry sectors will be limited due mainly to insufficient critical mass. However, it is anticipated that as the industry grows, the ability to undertake and/or invest in R&D using its own resources is equally expected to grow.

Government needs to continue on-going support to the industry through provision of expert assistance and advice, such as provided by extension services, and with export market development. Government has a role in supporting the industry through other means as well. These include providing reliable and streamlined licensing and permitting processes, and clearly defined and consistent regulations and enforcement processes, promoting aquaculture, countering negative views and encouraging corporate investment. R&D must be directed towards solution of practical problems and impediments facing the industry. Government aquaculture managers, research providers and industry need to recognise that not all problems have technical solutions, some problems warrant the efforts of organisations and institutions at the level of policy formation to create a climate that fosters industry development.

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APPENDIX I: INTELLECTUAL PROPERTY

The intellectual property (IP) gained from this project is in the form of a cumulative database and associated publications (Appendix III) relevant to the culture of Murray cod. The IP will be shared equitably between state agencies and access to the database and associated publications is available to relevant industry groups and other beneficiaries. The database will be managed by the Department of Primary Industry (PDI). There is no charge for database management and no patents were developed during the course of the project.

APPENDIX II: STAFF EMPLOYED ON THE PROJECT

Primary Industries Research Victoria (DPI)

Mr. Geoff Gooley

Dr Brett Ingram

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Mr Denis Lovric

Mr G.M. Turchini

APPENDIX III : PUBLICATIONS, REPORTS AND PRESENTATIONS EMANATING FROM THIS PROJECT

The following publications, reports and presentations have emanated from the Murray cod project:

Publications

Abery, N., Gunasekera, R.M. and De Silva, S.S. (2002). Growth performance of Murray cod *Maccullochella peelii peelii* (Mitchell), fingerlings fed diets substituted with varying levels of soybean meal and blood meal. *Aquaculture Research* 33: 279-289.

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Murray cod aquaculture production, along with 9 other DNRE projects, was featured in a static display at the Department of Natural Resources and Environment (DNRE) Annual Science Awards (22 February 2002).