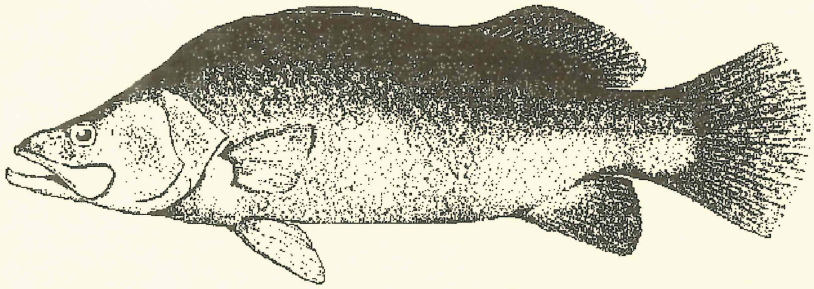
Larval and Juvenile Culture of Barramundi *Lates calcarifer* (Bloch)



Fishing Industry Research and Development Council

Project 89/67

FINAL REPORT

C.G. Barlow and M.A. Rimmer

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Submitted September 1993

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EXECUTIVE SUMMARY

This research project targeted specific aspects of barramundi larval and juvenile culture phases which were identified as restricting the continued development of barramundi aquaculture in Australia.

A major bottleneck in the intensive larval rearing of barramundi has been a mortality syndrome associated with a deficiency of highly unsaturated fatty acids (HUFAs) in the live prey organisms used for larval rearing (rotifers and brine shrimp nauplii). This syndrome was experimentally induced in barramundi larvae fed brine shrimp nauplii deficient in HUFAs. The mortality syndrome became apparent about 20 days after hatching and resulted in almost total mortality of barramundi larvae within 10 days. Evaluation of various nutritional enhancement techniques indicated that both indirect enhancement of live prey organisms to increase their HUFA content and direct enhancement (that is, feeding zooplankton species which contain naturally high levels of HUFAs) had potential application in barramundi larval rearing.

The results of experimental assessment of these various techniques provide a variety of enhancement procedures for hatchery managers. The most simple and effective technique is indirect enhancement, which entails feeding live prey organisms with microencapsulated diets prior to the organisms being eaten by the barramundi larvae. The microencapsulated diets are relatively inexpensive, are easy to store and handle, and are readily available commercially. Alternatively, direct nutritional enhancement by feeding copepods (which are high in HUFAs) can be undertaken, although this technique requires some additional hatchery facilities dedicated to copepod culture. The research also showed that the use of inert (microparticulate) diets holds little promise for use with barramundi, although further research on these diets, and the rearing procedures used in conjunction with inert diets, may improve the acceptability of inert diets for barramundi larvae. The use of microencapsulated diets to increase the HUFA content of live prey organisms used in aquaculture has become widespread in Australia, due in part to the results obtained by this research project.

Research undertaken on feeding activity at different photoperiods showed that photoperiod effects are more pronounced in smaller fish, particularly larvae. Increased photoperiod for larval rearing resulted in increased growth rates, but did not improve survival. In contrast, increased photoperiod for juvenile barramundi resulted in greater food consumption rates but no improvement in either growth or condition.

The nursery phase, particularly the weaning procedure, has long been regarded as a major bottleneck in barramundi culture. This research project showed that

larger barramundi fry wean more readily to pellet feeds and achieve greater survival than do smaller fry. Survival rates of over 90% during weaning are possible with fry 16 mm TL or larger, and similar results should be readily achieved by commercial aquaculture operations.

Assessment of the effects of fish density on grow-out of juvenile (< 60g) barramundi showed that growth rates are maximised at higher densities (240-360/m^3). These results provide barramundi farmers with several management options which can be used to maximise growth rate or production according to market demand and production schedules.

Differences in growth rates between different genetic strains of barramundi have been postulated on the assumption that cold-acclimated strains from southern Queensland would grow faster when translocated to warmer conditions in northern Queensland. Assessment of growth and survival rates between three strains of barramundi, from the Burrum River (south-eastern Queensland), Cairns (north-eastern Queensland), and Weipa (north-western Queensland), indicated that differences in growth between the separate strains were relatively minor and unlikely to be of consequence to barramundi farmers. In contrast, higher mortality of Cairns strain barramundi (32%) compared with Weipa strain barramundi (2%) was evident when pond temperatures were less than 20°C. Pond based farming of barramundi is not recommended in areas where pond temperatures regularly drop to below 20°C. Based on these results, the use of different strains of barramundi is unlikely to offer any immediate advantages in aquaculture.

Overall, the results of this research were successful in overcoming major production bottlenecks in the larval rearing and nursery phases of barramundi aquaculture, and provided detailed information on management procedures which can be used to increase the efficiency of all phases of barramundi aquaculture, including grow-out. Because of the relatively advanced stage of development of the barramundi aquaculture industry, many of the techniques developed during this project have found application in the development of aquaculture techniques for other native fish species throughout Australia.

OUTCOMES FROM FIRDC PROJECT 89/67

Increased availability of barramundi fingerlings for farmers due to enhanced survival and growth rates.

Development of larval rearing procedures for barramundi which prevent the occurrence of the mortality syndrome associated with deficiencies of highly unsaturated fatty acids:

Enhanced growth rates for intensively reared barramundi larvae due to better nutrition:

Enhanced growth rates for intensively reared barramundi larvae due to definition of optimal environmental conditions:

Improved survival during the nursery phase.

Definition of optimal weaning procedures and relationship between fish size and weaning success:

Increased efficiency of barramundi grow-out operations.

Comparative survival and growth rates of three separate genetic strains of barramundi:

Definition of optimal densities to provide maximal growth rates in cage culture:

Enhanced knowledge of disease of barramundi.

Increased knowledge of the range of diseases encountered in freshwater and marine culture of barramundi:

Increased knowledge of prophylactic and therapeutic procedures to control disease in barramundi:

Increased availability of other finfish fingerlings due to the widespread adoption of nutritional enhancement techniques developed during this project.

Development of cost-effective nutritional enhancement techniques for live prey organisms used in marine fish larval rearing:

Development of culture techniques for marine copepods suitable for marine fish larval rearing.

EXTENSION OF RESEARCH RESULTS

The results of research undertaken during this project have been communicated to barramundi farmers, other aquaculturists, and researchers in Australia and overseas. Informal extension of these results was carried out throughout the project by regular communication with interested parties in the fields of industry and research. Informal extension was facilitated by undertaking some components of the project on a commercial farm.

Formal extension of results was undertaken by means of workshops which were organised to communicate our research results directly to industry, and by publication of results in the scientific literature and in industry magazines. These extension outcomes are listed below; other publications arising from this research will be published in the scientific literature and in industry magazines in the near future.

Barramundi producers on a tour of farms undertaken as part of the Barramundi Farming Workshop held at the Freshwater Fisheries and Aquaculture Centre, Walkamin, 23-25 September 1993.

WORKSHOPS

Barramundi Aquaculture and Stocking Workshop, held at the Northern Fisheries Centre, Cairns, December 1991.

Barramundi Farming Workshop, held at the Freshwater Fisheries and Aquaculture Centre, Walkamin, September 1993.

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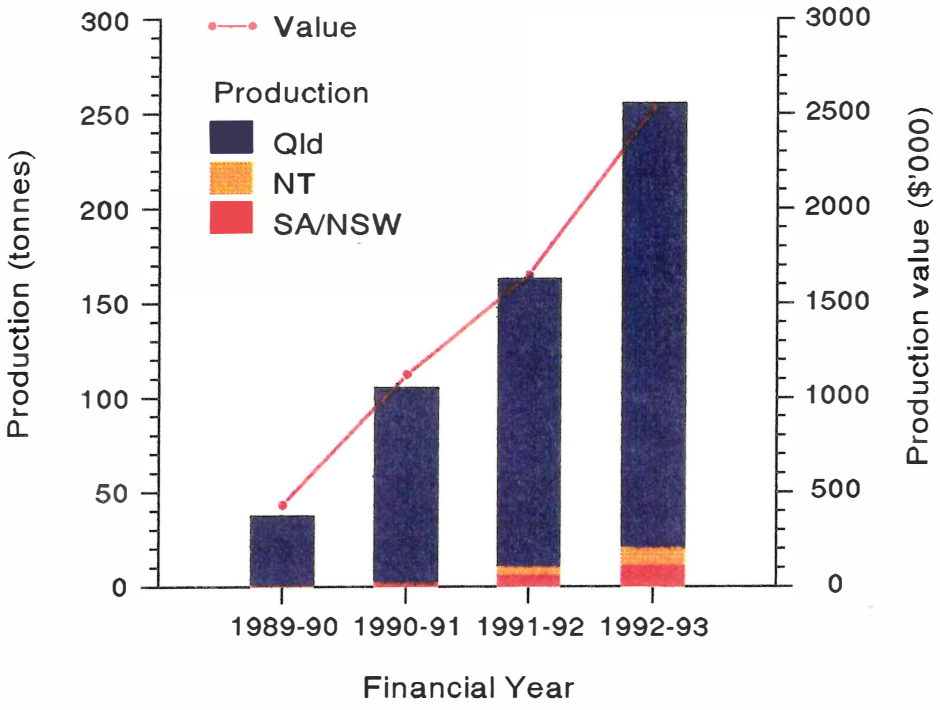
Chapter 1:

OVERVIEW OF THE BARRAMUNDI *Lates calcarifer* FARMING INDUSTRY IN AUSTRALIA.

Authors: C.G. Barlow and M.A. Rimmer



Production and value of Australian farmed barramundi in the years 1989-90 to 1992-93.

HISTORY OF BARRAMUNDI AQUACULTURE

Barramundi or Asian sea bass, *Lates calcarifer* (Bloch), is widely distributed in coastal and fresh waters throughout the Indo-west Pacific region. The limits of its distribution are Pakistan in the west, east to the east coast of northern Australia and north to the Chinese mainland (Greenwood 1976, Grey 1987). Throughout its range it is important in commercial fisheries and in the last two decades it has become prominent as an aquaculture species.

There are early reports of farming of barramundi using wild caught fingerlings in India (Menon 1948), and undoubtedly pond-rearing of wild progeny was practised in other South-east Asian countries. However, large-scale farming was only possible after controlled breeding was realised in Thailand in the early 1970's (Wongsomnuk and Manevonk 1973). Thereafter, the species was successfully propagated in the Philippines (Harvey *et al.* 1985), Taiwan (Lin *et al.* 1985), Singapore (Lim *et al* 1986) and Malaysia (Ali 1987). Barramundi now forms the basis of well developed farming operations in these countries.

In Australia, detailed work on barramundi culture was instigated in 1983, in two separate projects. The first was a project on development of barramundi hatchery and farming techniques, funded by the Fishing Industry Research Trust Account (FIRTA Project 83/38). This project was conducted in the Cairns-Innisfail region in north Queensland, and led to the formation in 1985 of the publicly listed aquaculture company 'Sea Hatcheries'.

The second was a program conducted by the Queensland Department of Primary Industries (QDPI) at its Cairns and Walkamin laboratories. The initial aims of the work were to investigate controlled breeding and production of fingerlings for stocking freshwater impoundments in north Queensland. The program was given high priority within QDPI, because if successful, the use of barramundi for enhancing recreational fisheries would provide an alternative to the exotic Nile perch, which was concurrently being investigated with a view to importation and stocking in northern Australian waters.

Both the FIRTA and QDPI projects were successful in establishing controlled breeding of barramundi (Heasman *et al.* 1985, MacKinnon 1987), and the resultant supply of fingerlings led to the establishment of grow-out farms in northern Queensland in the late 1980's. About the same time, several private hatcheries commenced operation. The capability of the industry to produce fingerling barramundi was dramatically improved with the development of larval pond-rearing techniques (Rutledge and Rimmer 1991) and the sale of fertilised eggs by QDPI, which commenced in 1990. These advances allowed barramundi farmers to produce

their own fingerlings, rather than relying on production by the larger hatcheries.

Outside Queensland, the Northern Territory Department of Primary Industry and Fisheries started a program on barramundi aquaculture in 1987. Six grow-out farms are now licensed in the Northern Territory. In South Australia, West Beach Aquaculture Pty Ltd has established a hatchery operation, which provides fingerlings, expertise and facilities to a network of grow-out farms (thus far, in New South Wales and South Australia). There is also one farm in South Australia producing barramundi in geothermal water.

TYPES OF FARMING SYSTEMS USED IN AUSTRALIA

Broodstock Maintenance

Early work on culture techniques for barramundi relied heavily on obtaining fertilised eggs by stripping running-ripe male and female barramundi caught on spawning grounds. This approach is expensive and unreliable and has now largely been replaced by the development of controlled breeding techniques for captive broodstock. Note, however, that limited access to wild fish is still required to replace broodstock and to provide male fish (males change to females in captivity, as in the wild).

Purpose built systems are required for broodstock maintenance. The fish are usually held indoors, in either flow-through or recirculating systems. Barramundi broodfish may be kept in either fresh or salt water but must be placed in salt water prior to the breeding season to enable final gonadal maturation to take place. The ripe broodstock fish are injected with reproductive hormones which induce the fish to spawn, usually at dusk, about 36 hours after injection. The Northern Fisheries Centre, Cairns, has recently bred barramundi throughout the year using controlled environment systems to manipulate temperature-photoperiod cycles, thus enabling spawning outside the normal spawning season.

Larval-fingerling rearing

Barramundi larval rearing is undertaken using either 'intensive' or 'extensive' techniques. Intensive larval rearing involves the culture of larvae in a controlled environment, such as a hatchery, where the fish larvae are supplied with prey organisms which are also cultured under controlled conditions. The intensive system requires dedicated facilities and a high degree of technological skill. In contrast, extensive larval rearing involves the culture of larvae in a largely uncontrolled environment (a pond) and the culturist has relatively little direct control over factors such as water quality, prey organism density and disease.

In Asia, barramundi larvae are reared intensively, and it was these larval rearing techniques which were originally introduced to Australia where they are still used in a few hatcheries. However, the majority of barramundi fry now produced in northern Australia are produced using extensive larval rearing procedures.

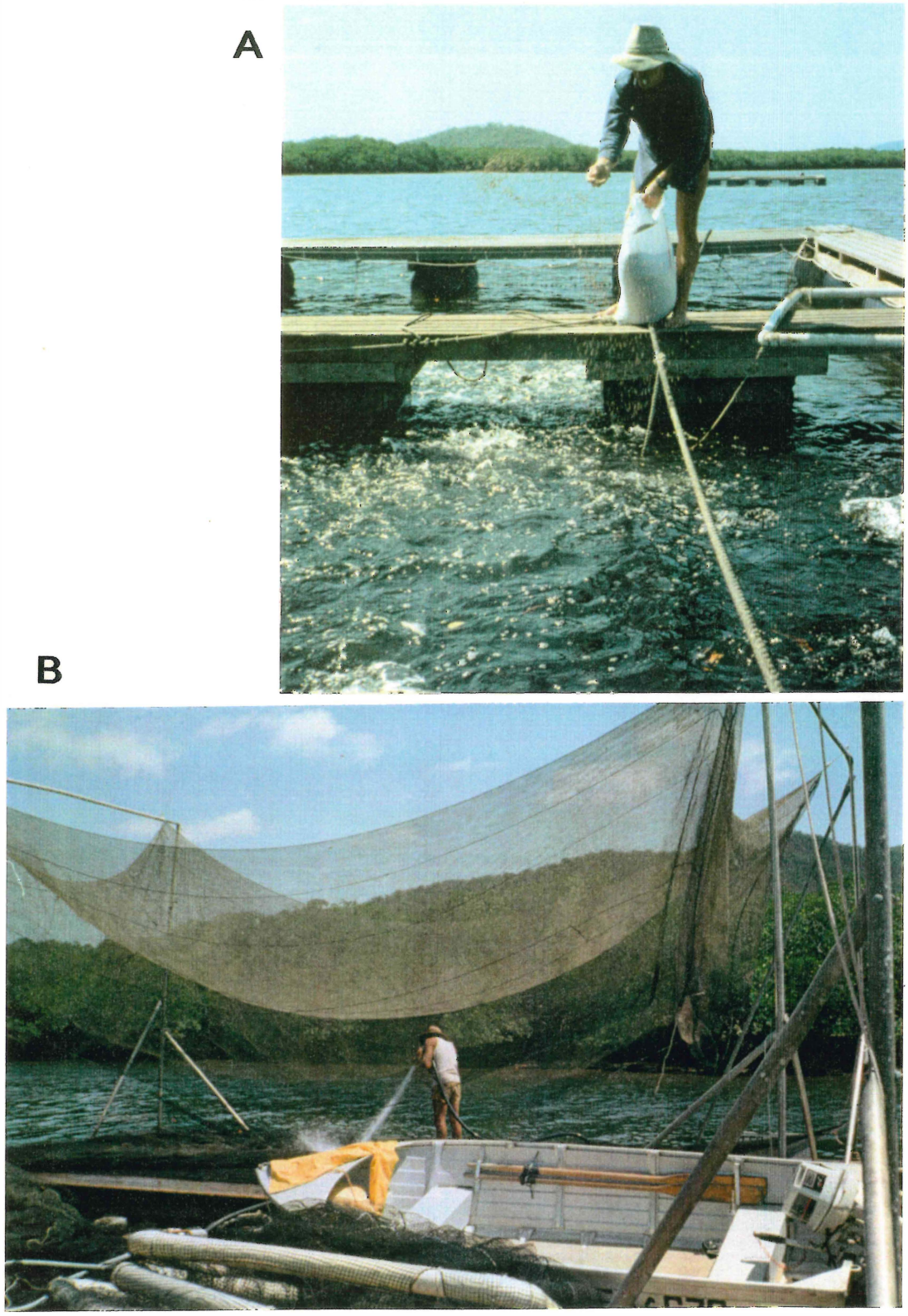
Barramundi can be weaned onto inert or pelleted diets at a relatively small size, although the ease and success with which weaning can be accomplished depends primarily on the size of the barramundi. Weaning can be initiated with fish as small as 10 mm total length, but it is much more successful with fish 16 mm or larger.

Grow-out systems

There are three quite different methods currently used in Australia for growing weaned fingerlings to market size. One is cage culture in estuarine waters. Relatively few companies are using this technique. One is Sea Harvest Pty Ltd (formerly Sea Hatcheries), which is the biggest producer of farmed barramundi in Australia. Cage culture in estuarine or marine waters has considerable advantages over other systems, particularly where large-scale production (several hundred tonnes or more per annum) is envisaged. There are, however, problems with biofouling and to a lesser extent predators.

The most common grow-out system in Australia is pond culture, in either brackish or freshwater. Fish are usually maintained in cages, although nowadays cage culture of fish less than 120-150 mm total length and free-ranging for larger fish are sometimes combined. Pond rearing of free-ranging fish does not require the labour associated with cage culture, and produces fish with a better appearance and colour (silver rather than dark grey to black). The major disadvantage of this method is difficulties in stock management and harvesting.

The third method of farming barramundi is intensive production in an indoors, controlled environment building, using bore water (ie., pathogen free) and a high level of recirculation through biological filters. This facility and technology have been developed and patented by a South Australian company (West Beach Aquaculture Pty Ltd), which is currently establishing plants in South Australia and New South Wales. The facility is generic, in that it is suitable for any species which can be intensively farmed. Because of the controlled environment, it allows for year-round production virtually anywhere that bore water is available, regardless of the temperature requirements of the species.



Feeding barramundi (A) at Sea Harvest's farm, situated in an estuary near Cardwell. Biofouling is a major problem in salt water, necessitating constant changing and cleaning of nets (B).

Most barramundi farms are based on fresh water ponds, in which the fish are usually maintained in cages, although some producers also free-range fish in the ponds. This farm is located near Cairns. The ability of the species to thrive in salt and fresh water widens the options for siting of farms.

BARRAMUNDI PRODUCTION IN AUSTRALIA

Production of barramundi from aquaculture in Australia commenced in 1986, with 2 tonne of fish coming from Sea Hatcheries' farm in that year. Production has increased significantly since then, with approximately 235 tonnes (live weight) being sold from Queensland farms in 1992/93 (Table 1.1).

In addition to Queensland, farmed barramundi are now being produced in the Northern Territory, South Australia and New South Wales. There is also one pilot-scale operation in Lake Argyle, Western Australia. Considering the increasing production from aquaculture and the decreasing value of the capture fishery (Table 1.1), it is conceivable that the value of the barramundi aquaculture industry will approximate that of the capture fishery within a few years.

Table 1.1

Production and value of barramundi from aquaculture and capture fisheries in Australia in the financial years 1989/90 to 1992/93. Production figures are tonnes live weight. t = tonnes. N.A. = not available at the time of printing.

FISHERY	STATE	1989/90 t	1989/90 $'000s	1990/91 t	1990/91 $'000s	1991/92 t	1991/92 $'000s	1992/93 t	1992/93 $'000
AQUACULTURE	Qld[a]	37	429	103	1100	152	1538	235[g]	2313[g]
	NT[b]	0	0	0	0	5	51	10	100
	SA/NSW[c]	0	0	2	22	5	55	10	110
	TOTAL	37	429	105	1122	162	1644	255	2523
CAPTURE FISHERY	Qld[d]	733	5864	783	3913	780	3900	N.A	
	NT[d]	550	2052	459	1819	460	1817	N.A	
	WA[e]	56	346[f]	61	282[f]	46	212[f]	N.A	
	TOTAL	1339	8262	1303	6014	1286	5929		

Information sources:

a. Aquaculture Production Surveys 1989-90, 1990-91, 1991-92. Reports published by Queensland Department of Primary Industries.
b. Personal communication from Dr. C. Shelley, N.T. Department of Primary Industries and Fisheries.
c. Personal communication from Dr. J. Trendall, West Beach Aquaculture Pty Ltd.
d. Australian Fisheries Statistics 1992. Aust. Gov. Publishing Service.
e. Personal communication from Ms. H. Brayford, W.A. Fisheries Department
f. Estimated from value of QLD and NT product.
g. Preliminary estimate from early production returns.

ROLE OF RESEARCH IN INDUSTRY DEVELOPMENT

The barramundi aquaculture industry in Australia would not have developed without the applied research conducted by both government and private agencies. Despite the fact that much published literature on barramundi farming in other countries is available, virtually all of the major advances which have taken place here have been the result of local research. In fact, the barramundi farming industry is a good example of the almost universal need for local research to adapt overseas technology before it can be applied in Australia.

This requirement for local research is evident in several areas within the industry. For instance, techniques necessary for broodstock maintenance and spawning in Australia have proven to be considerably more complex than those used in south-east Asia.

Sex-reversal is a complicating factor in Australia, necessitating continual broodstock replacement, but in south-east Asia sex-reversal is not a common phenomenon. Breeding in captivity in Australia is seasonal and requires the application of hormones to stimulate spawning, while in south-east Asia spawning takes place for 8-10 months, and natural spawning in broodstock tanks is common.

'Out of season' or year-round spawning of barramundi has recently been achieved at the Northern Fisheries Centre, Cairns. Year-round spawning requires facilities in which temperature and photoperiod can be controlled. Its major advantage is that it enables producers to access fingerlings at the start of the growing season, thus making possible production of plate sized product without having to hold the fish through an over-wintering period.

Major advances in barramundi larval nutrition have been made in Australia as a result of research carried out as part of this project (FIRDC 89/67). The research was novel in Australia, and highlighted the need for adequate food quality (not just quantity) in intensive larval production of other species. As a result, nutritional supplementation of live feeds is now a common practice with intensive larval production of a range of species in Australia.

Probably the most significant development in barramundi production in Australia has been the use of brackish water ponds for larval rearing. Initial larval rearing techniques followed south-east Asian intensive hatchery production systems, but labour costs made this an expensive technique in Australia. The use of brackish or marine ponds for larval rearing is practised in Texas, USA, for mass production of fingerling fish for enhancement of coastal fisheries. This rearing system for barramundi larval production was evaluated using a brackish water pond on a prawn farm in north Queensland. The research proved extremely successful, as the pond system did not require a high degree of expertise or infrastructure, and resulted in large numbers of fingerling fish (Rutledge and Rimmer 1991). The system effectively lowered the cost of fingerlings from about 50 cents to 10-15 cents each,

which had a significant benefit for barramundi farming (Treadwell *et al.* 1991). It is now routinely used by farmers in Queensland and the Northern Territory.

Another important area where research is continually being undertaken and new techniques developed is on the farm. As barramundi farming is a comparatively new activity, there is not a large bank of knowledge on farm management practices. Consequently, producers are regularly testing new systems and procedures to improve their efficiency. This area of research, although often not acknowledged, is equally as important for industry development as the more formal research undertakings of the scientific community. Obviously the greatest benefit will be attained if both sectors maximise their interaction.

Publications arising from research on barramundi aquaculture in Australia are listed in Appendix 1.1.

FUTURE RESEARCH AND INDUSTRY DEVELOPMENT NEEDS

Definition of research and industry development requirements within an industry must primarily come from industry personnel. In this report, we have not specifically attempted to collate such information. Nevertheless, we have provided comment on some current research and development issues facing the barramundi aquaculture industry, based on our experience with the industry in north Queensland. The list provides a focus for discussion of research requirements, but we stress that it is not definitive, nor does it address the broader development needs (eg., government services and regulation, access to finance) which are vitally important for industry development.

1. Breeding and Reproduction

- There is a need for industry to become self-sufficient in the supply of seed (fertilised eggs), so that there is no dependence on seed supply from government facilities as is the case at present. This will necessitate some operators within the industry developing broodstock maintenance and spawning facilities.

2. Larval and Nursery Rearing

- Further research is required on the extensive rearing of larvae in ponds to define parameters affecting survival, and management procedures to improve the reliability of this technique.

- Nursery rearing appears to be an area where there is excessive mortality, as the large number of fingerlings being produced in larval rearing ponds is not resulting in a proportionally large number of juvenile fish on grow out farms. More information is required on farm-based systems, water quality

management, feeding procedures and disease control. The QDPI conducted a workshop in September 1993 to address this issue, but it is probable that targeted research and/or extension will be required to significantly improve survival in nursery operations.

3. Grow-out Systems

- Access to marine sites for cage culture is only a minor issue at present. However, there is little doubt that if fin-fish aquaculture in tropical Australia is to become a major industry (comparable to salmon in Tasmania or tuna in South Australia) access to marine sites will be necessary. This will require aquaculture needs to be catered for in coastal zone planning.

- Techniques for cage culture in marine waters will need to be refined, particularly in areas of site selection, cage design, control of biofouling and predator protection. Considerable information on most of these aspects is available in temperate Australia, although site selection and management in the tropics may be considerably different.

- Definition of optimum techniques for pond culture in both fresh and brackish waters is still required. Economic analyses of industry experiences with cage culture in ponds, free ranging of fish and combination of both would help in this regard.

- Mechanisation of grading, harvesting, counting and transport procedures would be beneficial, particularly on large farms. Equipment to handle these procedures is available for salmonid fish, but some modification would be required to adapt it for use with barramundi.

- Environmental aspects of farm design and management, particularly in coastal areas, is an issue pertinent to industry development. In fresh waters, mechanisms to remove nutrients from waste water (eg., terrestrial irrigation) prior to the water entering streams should be encouraged. In brackish waters, research is required to determine effective methods for removing nutrients (eg., settlement ponds, uptake via mangrove forests) before the water is released to estuaries.

4. Nutrition

- Nutritional research on barramundi aquaculture is currently being addressed within the FRDC-funded project at Walkamin (FRDC project 92/63), and the FRDC program on fish meal replacement in fish feeds. One specific aspect which may warrant further investigation is development of 'environmentally friendly' diets, that is, diets which result in minimal excretion of solid material and dissolved nutrients.

5. Fish Health

- Development of vaccines for certain bacterial diseases, specifically columnaris disease and *Vibrio harveyi* vibriosis, is a high priority.

- The fate and metabolism of chemotherapeutic agents used in treating diseases of barramundi need to be determined. The National Registration Authority will soon introduce specific registration requirements for drugs used in Australian aquaculture.

- Research on the pathophysiology of barramundi is still required to understand how stress affects disease incidence, growth and food conversion ratios. The provision of clean and 'natural' environments will have important animal welfare considerations.

6. Marketing

- The size of the Australian market for fresh, plate sized barramundi is unknown. Dedicated market research is required to determine the size and factors affecting demand. This information is essential to avoid a sharp drop in prices as output rises.

- When output is sufficient to satisfy the local demand for plate sized barramundi, it will be necessary for the industry to adopt value adding practices if prices are to be maintained. One method of value adding is already being evaluated through a FRDC-funded project (FRDC project 93/184) on live fish transport. Undoubtedly there is huge scope for development of processed products, such as pre-cooked packs, vacuum packing, smoking, etc., which would allow the products to be retailed through gourmet supermarket lines.

- Many farmed barramundi are very dark in colour. These fish are less preferred in the market than silver coloured barramundi. Methods to lighten dark fish prior to marketing would be beneficial to the industry, as would definition of the on-farm conditions which produce silver fish.

- There has been no promotion of farmed barramundi in the market place. The ability of producers to market their product would be enhanced by promotional campaigns aimed either specifically at farmed barramundi or more generally at aquaculture products.

- Coordination of marketing at an industry level would benefit all producers in the industry.

7. Diversification

- Given the considerable advantages (numerous species, access to markets, climate) that tropical Australia has for aquaculture of fin fish, there is no doubt that the industry can become a major generator of wealth and jobs in the region. However, for this to happen it will be necessary for production techniques to be established for a range of highly-valued species, as an industry reliant on one species is overly susceptible to the vagaries of the market place. The barramundi industry in north Queensland recognises this, and is highly supportive of breeding studies currently being conducted by QDPI on mangrove jack (*Lutjanus argentimaculatus*). Like barramundi, mangrove jack can be grown in both salt and fresh water, which widens the options for siting of farms.

The great majority of Australian farmed barramundi are sold as gilled-and-gutted, plate-sized product. As production increases, the industry will have to look at value-adding to maintain prices. This will require post-harvest and marketing research on barramundi, and diversification into other species.

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APPENDIX 1.1

PUBLICATIONS ON AQUACULTURE OF BARRAMUNDI IN AUSTRALIA

(* Publications arising from the Northern Fisheries Centre, Cairns, and the Freshwater Fisheries and Aquaculture Centre, Walkamin)

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Chapter 2

REVIEW OF THE NUTRITIONAL ASPECTS OF INTENSIVE REARING OF MARINE FISH LARVAE

Author: M.A. Rimmer

ABSTRACT

The literature pertaining to the nutritional requirements of marine fish larvae is reviewed in order to identify strategies which have been employed to enhance the nutritional profile of the diet of cultured larval fishes. Although the precise nutritional requirements of marine fish larvae are poorly known, mortality syndromes in several species have been shown to be associated with deficiencies of highly unsaturated fatty acids, particularly 20:5*n*-3 and 22:6*n*-3, in the diet. Nutritional enhancement strategies have been developed to increase the dietary levels of nutritional factors identified as essential to fish growth and survival. These strategies fall into two basic types: direct supplementation, in which the supplement is fed to the fish larvae; and indirect supplementation, in which the supplement is fed to the live prey organisms used to feed the fish larvae.

INTRODUCTION

The rearing of marine fish larvae requires a source of food which is suitable for larval survival and growth throughout the period of larval development. The most important criteria which must be met by any foods used are: adequate density to allow larvae to find food without extensive searching; contrast against the background to enable the larvae to sight the food; suitable movement to elicit a feeding ('strike') response; and suitable size for ingestion. In order to meet these criteria, barramundi, and most other marine fish larvae, are fed on live zooplankton reared in the hatchery: rotifers, *Brachionus plicatilis*, and brine shrimp, *Artemia* spp. (Fig. 2.1). Barramundi larvae are initially fed on rotifers (ât a density of 10-20 rotifers/ml) from the commencement of feeding (day 2, where day 1 is the day of hatching) until day 14, and on brine shrimp (2-10 brine shrimp/ml) from day 10 until metamorphosis at about day 20 (Fig. 2.2).

Rotifers and brine shrimp are widely used for marine fish larval rearing because of their ease of culture. However, both organisms are now recognised as being deficient in several important nutritional components, particularly highly unsaturated fatty acids (HUFA's). Deficiencies of HUFA's in the live food organisms used for intensive larval rearing have been implicated in the larval mortality syndromes seen in a number of marine fish species, including barramundi (Rodgers and Barlow 1987, Rimmer *et al.* 1988, Dhert *et al.* 1990).

This review details the nutritional requirements of marine fish larvae and the nutritional problems associated with the use of live prey organisms used for intensive larval rearing.

NUTRITIONAL REQUIREMENTS OF FISH LARVAE

Although there have been numerous studies on the nutritional components of live foods for fish larvae, there is little published data on the precise nutritional requirements of marine fish larvae. This is at least partly attributable to the difficulty in experimentally manipulating diets to test each nutritional factor independently of other factors.

The majority of studies on nutritional components of larval fish foods have indicated that fatty acids, particularly long chain fatty acids of the *n*-3 series, are the major limiting factors in larval fish nutrition (Watanabe *et al.* 1983, 1989). However, Dendrinos and Thorpe (1987) found one amino acid (proline) to be present in substantial amounts in the egg yolk of sole (10.89 g / 100 g protein) but present in only trace amounts in the rotifers and brine shrimp used as foods, and suggested that this may indicate a nutritional deficiency.

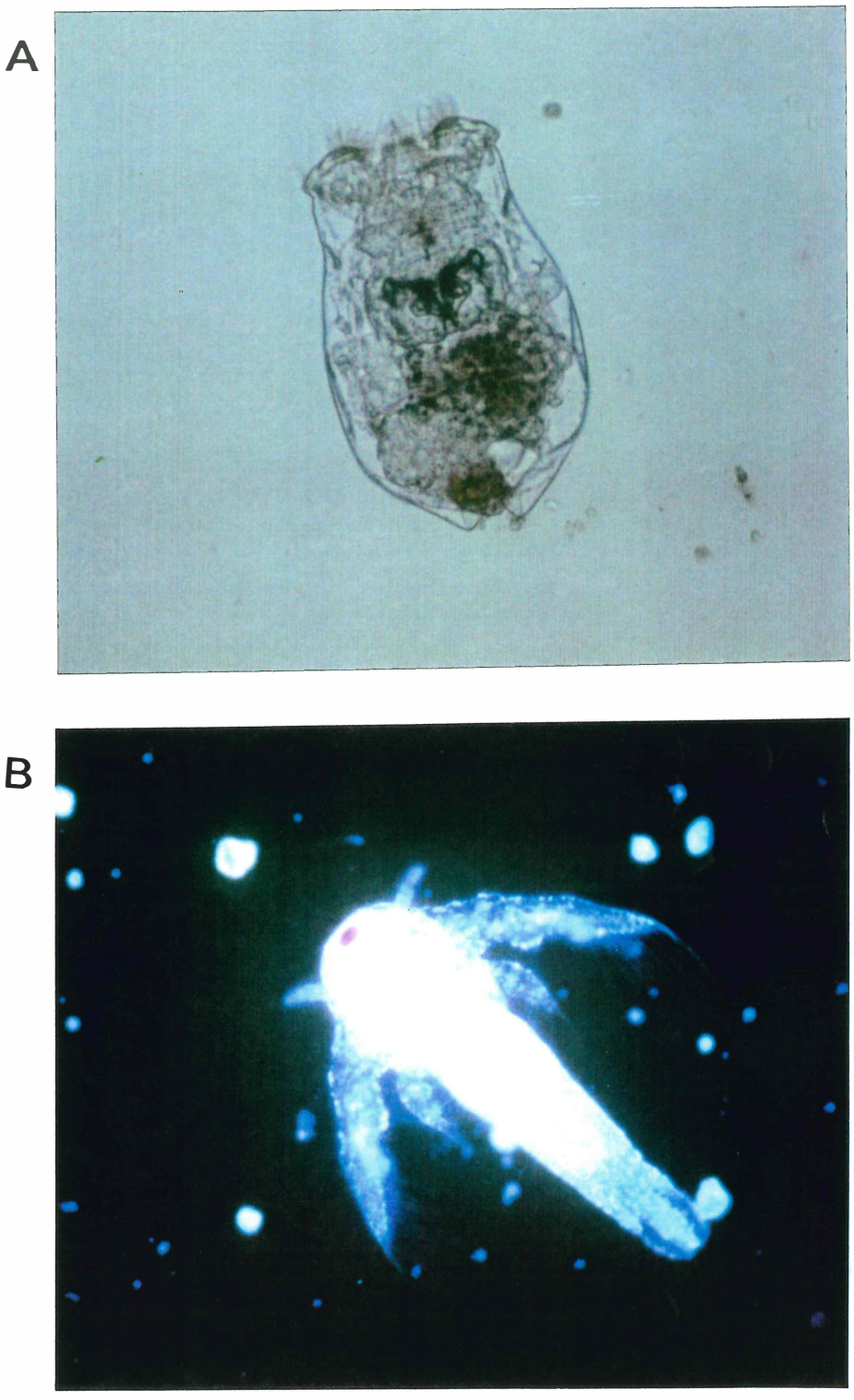

Figure 2.1. Live food organisms used in the intensive larval rearing of marine fishes: (a) rotifer, *Brachionus plicatilis*, and (b) brine shrimp, *Artemia* sp.

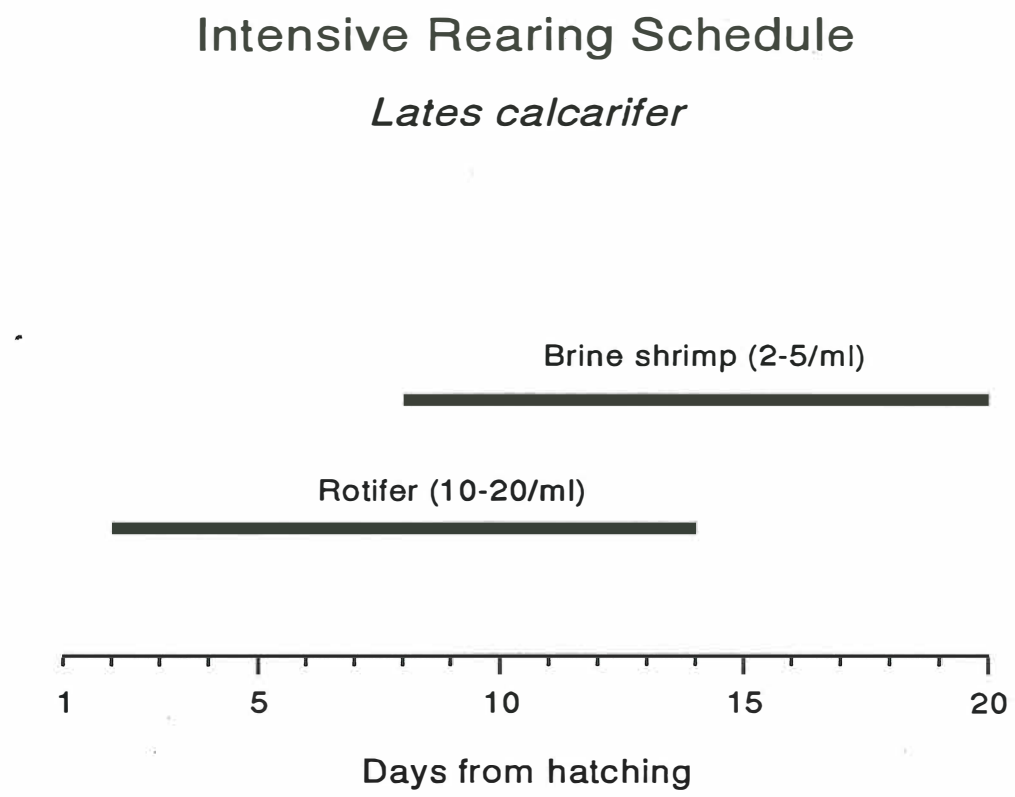

Figure 2.2. Schedule for feeding rotifers and brine shrimp to barramundi larvae reared intensively.

Fatty acids are important components of the biomembranes in fish (Cowey and Sargent 1972). The predominant fatty acids in fish tissues are in the *n*-3 series such as linolenic (18:3*n*-3), eicosapentaenoic (20:5*n*-3) and docosahexaenoic (22:6*n*-3) (Cowey and Sargent 1972, New 1986). (See Appendix 2.1 for an explanation of fatty acid nomenclature). In comparison, the tissues of terrestrial animals are high in *n*-6 fatty acids, such as linoleic (18:2*n*-6) and arachidonic (20:4*n*-6) (New 1986).

The essential fatty acids (EFA's) in marine fish are generally considered to be the C20 and C22 HUFA's, particularly 20:5*n*-3 and 22:6*n*-3 (Watanabe *et al.* 1983, 1989, New 1986). However, survival in some species may also be limited by levels of C18 fatty acids (Dendrinos and Thorpe 1987).

The requirement for *n*-3 HUFA's in marine fish larvae ranges from 0.5% - 2.0% of total fatty acids (Watanabe *et al.* 1989), although these requirements vary between species and may also vary between different growth stages in the same species (Izquierdo *et al.* 1989). Cowey and Sargent (1972) describe the major symptoms of fatty acid deficiency as the occurrence of a 'shock syndrome' in which fish swim in an erratic fashion and then 'faint' to either die or recover; depigmentation of the skin; and erosion of the skin and fins. Low levels of *n*-3 HUFA's have also been associated with increased incidence of disease (Watanabe *et al.* 1989, Corneillie *et al.* 1990) and decreased rates of survival and growth in a range of marine fish species (Witt *et al.* 1984, Dendrinos and Thorpe 1987, Sorgeloos *et al.* 1988, Watanabe *et al.* 1989, Dhert *et al.* 1990).

Although all living organisms can synthesise *de novo* saturated and monounsaturated fatty acids, only photosynthetic organisms are able to synthesise *de novo* HUFA's (Sargent 1976). Fish larvae obtain HUFA's from three main sources:

1. Stored fatty acids in the lipid component of the yolk and oil globule;
2. By elongating and desaturating shorter chain fatty acids to produce HUFA's;
3. From prey animals or plants.

The first of these sources, HUFA's stored in the yolk and oil globule, is the sole source of fatty acids for fish larvae until the commencement of exogenous feeding. In the case of barramundi larvae, the yolk is absorbed rapidly over the first 24 hours after hatching and is almost completely used up by 50 hours after hatching (Kohno *et al.* 1986) (Fig. 2.3). The oil globule is absorbed more slowly and persists for about 140 hours after hatching (Kohno *et al.* 1986) (Fig. 2.3). Some authors have defined the nutritional requirements of fish larvae as being the equivalent of the nutritional composition of the yolk, on the assumption that the yolk provides a complete 'diet' for the yolk-sac larva (Dendrinos and Thorpe 1987, Kanazawa *et al.* 1989, Corneillie *et al.* 1990). While this assumption is probably reasonable for newly hatched larvae, it should be noted that larval development is a dynamic process which includes the development of internal organs and their associated metabolic pathways and thus the nutritional requirements of larvae may change substantially as they develop (Dabrowski *et al.* 1984). For example, Dendrinos and Thorpe (1987) suggested that the requirements of sole larvae for 20:5*n*-3 and 22:6*n*-3 fatty acids during the first

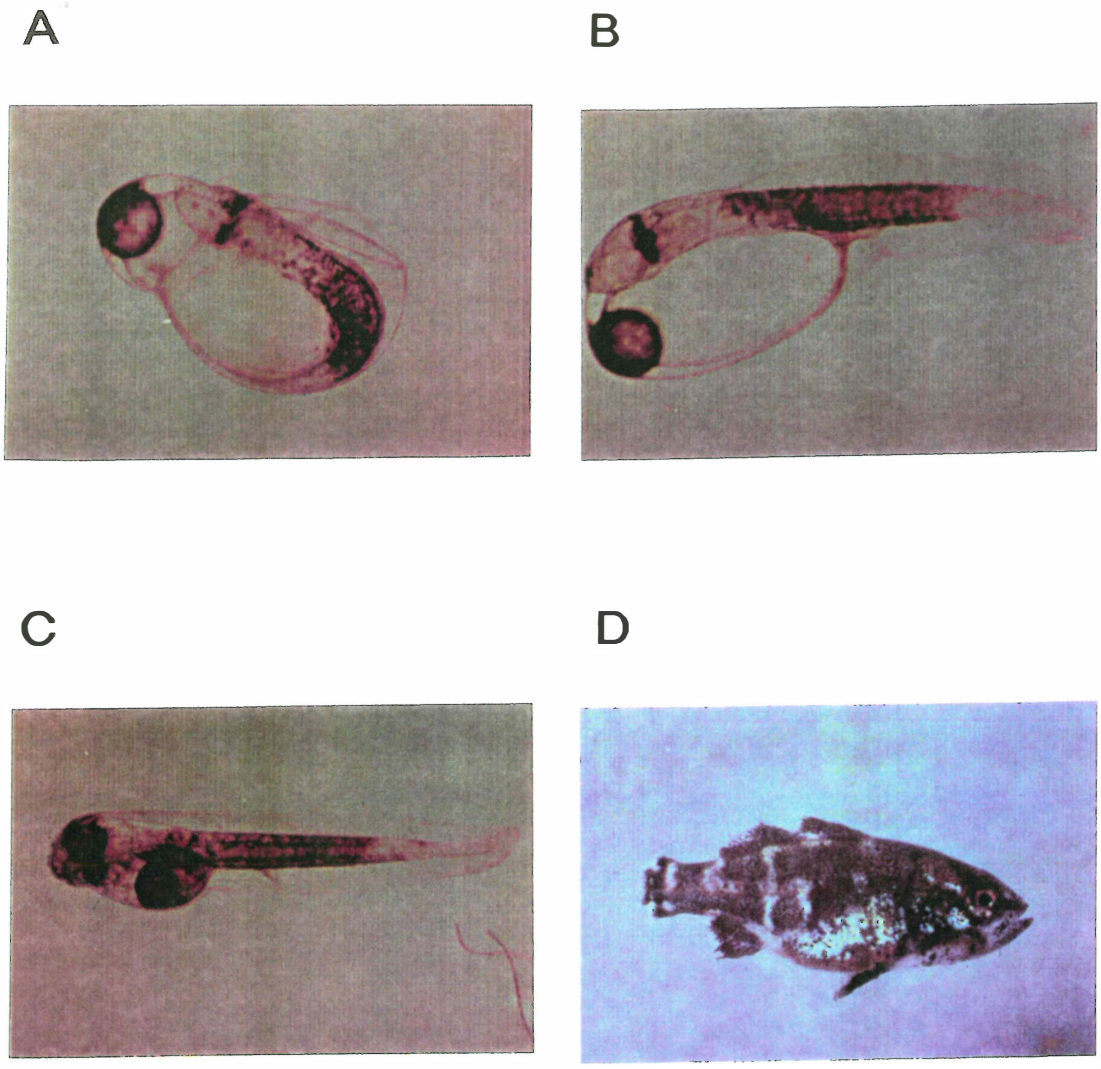

Figure 2.3. Stages of larval development of barramundi.
(a) Larva hatching from egg, 12-17 hours after fertilisation.
(b) Newly hatched larva. Note large yolk with anterior oil globule, lack of functional mouth and digestive system.
(c) First-feed larva, day 2 from hatching. The yolk has been absorbed, the mouth and gut are formed and the larva is ready to commence feeding.
(d) Juvenile barramundi, day 20 from hatching (10 mm TL). The fish has the adult complement of fin rays and scalation is complete.

few days of larval development could be met by the small quantities in the foods used, and that after this period these requirements could be met by elongation and desaturation of C18 fatty acids.

The relative importance of the second source of fatty acids for fish larvae, elongation and desaturation of shorter chain fatty acids, is poorly known. Marine percoid fish are less able to elongate and desaturate fatty acids than freshwater percoids (New 1986). As previously mentioned, the metabolic capabilities of fish larvae change substantially during early development and the ability of fish larvae to metabolise HUFA's may also change. In addition, the process of desaturation has been found to be temperature dependent in some fish species and may not take place at relatively low temperatures (Dendrinos and Thorpe 1987).

The third source of HUFA's for fish larvae, their prey, is discussed in detail in the following section.

NUTRITIONAL COMPOSITION OF LIVE FOOD ORGANISMS

Microalgae

The different types of microalgae cultured for use in intensive hatchery systems vary significantly in their nutritional composition (Brown *et al.* 1989). To date, much research has centred on the *Chlorella* and *Nannochloropsis* species which are commonly used in intensive aquaculture of marine fishes (Hirayama and Nakamura 1976, Scott and Middleton 1979, Watanabe 1979, Watanabe *et al.* 1983).

Freshwater *Chlorella* species are high in 18:3*n*-3 (an EFA for freshwater fish) but have low levels of 20:5*n*-3 (an EFA for marine fish), while marine *Chlorella* species (at least some of which have recently been found to belong to the eustigmatophyte genus *Nannochloropsis* [Anon 1990]) have low levels of 18:3*n*-3 and high levels of 20:5*n*-3 (Watanabe *et al.* 1983, Brown *et al.* 1989). Neither type contains significant amounts of 22:6*n*-3, but this fatty acid is found in reasonably high levels in golden-brown flagellates such as *Isochrysis* and *Pavlova* (Scott and Middleton 1979, Lubzens *et al.* 1985, Brown *et al.* 1989).

The precise nutritional composition of the microalgae used depends on culture techniques (particularly temperature), the phase of the growth cycle and the genetic strain of microalga used (Sargent 1976, Scott and Middleton 1979, Lubzens *et al.* 1985, Brown *et al.* 1989).

Nutritional deficiencies can be overcome by feeding a range of microalgae rather than a single species (Brown *et al.* 1989). However, large scale culture of many

microalgal species is difficult and unreliable. As a result, commercial hatcheries tend to rely on one or two easily cultured species, regardless of their nutritional value.

Yeasts

Several types of yeast have been used as food for rotifers and brine shrimp in intensive culture systems. The main advantage of yeast as a food source is that far less facilities are required compared with microalgal culture. However, yeasts are deficient in *n*-3 HUFA's, particularly 20:5*n*-3 and 22:6*n*-3 (Watanabe *et al.* 1983, Dendrinos and Thorpe 1987).

These HUFA's can be incorporated in the diet by culturing the yeast on a medium containing high levels of *n*-3 HUFA's, such as fish liver oils; such yeasts have been termed omega-yeasts (Watanabe *et al.* 1983).

Rotifers

The mineral composition of yeast-fed rotifers (*Brachionus plicatilis*) is largely unaffected by the mineral composition of the yeasts used as food (Watanabe *et al.* 1983). In contrast, the proximate composition of the food organisms is reflected in the proximate composition of the rotifers. Water content is greater in rotifers cultured on yeast than those cultured on microalgae, while lipid levels are higher in the latter (Watanabe *et al.* 1983). The lipid levels of yeast-fed rotifers directly reflect the lipid levels of the yeasts used as food (Dendrinos and Thorpe 1987).

The amino acid composition of rotifers fed on yeasts does not differ from those fed on microalgae (Watanabe *et al.* 1983, Dendrinos and Thorpe 1987).

The fatty acid composition of rotifers reflects the fatty acid composition of the foods used to rear them (Scott and Middleton 1979, Watanabe 1979, Watanabe *et al.* 1983, Lubzens *et al.* 1985, Dendrinos and Thorpe 1987). However, the relationship between fatty acid levels of rotifers and their food organisms is not always proportional. In particular, rotifers fed on a diet deficient in C20 and C22 HUFA's have been found to contain significant levels of 22:6*n*-3, suggesting that *Brachionus* is capable of elongating and desaturating fatty acids (Lubzens *et al.* 1985, Dendrinos and Thorpe 1987). However, the rate of synthesis of these HUFA's is low and the levels reached are well below the levels regarded as essential for adequate nutrition of marine fish larvae.

The nutritional composition of rotifers can be upgraded by several techniques:

1. Secondary culture of yeast-fed rotifers with microalgae; this technique has been shown to increase the levels of 20:5*n*-3 from trace amounts to a maximum of 27% after 2 days secondary culture with *Chlorella* (Watanabe 1979, Watanabe *et al.* 1983).

2. Indirect method: the levels of *n*-3 HUFA's in the rotifers can be increased by culturing yeasts on media incorporating fish liver oil (Watanabe *et al.* 1983, Dendrinos and Thorpe 1987).

3. Direct method: rotifers can be fed a homogenised mixture of fish oil and egg yolk (Fontaine and Pevera 1980, Watanabe *et al.* 1983), a liquid supplement (Sorgeloos *et al.* 1988) or microcapsules (Walford and Lam 1987). Maximum levels of HUFA incorporation are reached after 8 hours of secondary culture with microcapsules (Walford and Lam 1987).

Brine Shrimp

The nauplii of the brine shrimp, *Artemia* spp., are widely used as food for larval and juvenile fishes, primarily because of the convenience with which the resistant cysts can be stored for long periods before use.

Nutritional composition varies between different strains of brine shrimp. Japanese workers have classified *Artemia* strains into two types: a 'freshwater' type which is high in 18:3*n*-3 and a 'marine' type which has high levels of 20:5*n*-3. Neither type has high levels of 22:6*n*-3 (Watanabe 1979, Watanabe *et al.* 1983).

The water and lipid content of brine shrimp is highest in newly hatched nauplii (Dendrinos and Thorpe 1987). Unfed brine shrimp nauplii show a number of changes in their nutritional composition during starvation. Lipid levels initially decrease and protein levels increase concomitantly, followed by a decrease in protein as carbohydrates, lipids and proteins are sequentially utilised as energy sources. In addition, the level of 18:3*n*-3 fatty acid decreases and 20:5*n*-3 and 22:6*n*-3 levels increase, presumably as a result of elongation and desaturation of C18 fatty acids (Watanabe 1979, Dendrinos and Thorpe 1987).

Brine shrimp can be fed on yeasts, microalgae or particulate artificial feed to increase the size of the food organisms or to supplement existing nutritional components (Jones *et al.* 1974, Sakamoto *et al.* 1982, Walford and Lam 1987). The proximate biochemical composition of yeast-fed brine shrimp is largely

affected by the biochemical composition of the yeast supplied (Dendrinos and Thorpe 1987).

Similarly, the fatty acid composition of brine shrimp fed on yeasts or microalgae reflects the fatty acid composition of the foods used, as well as biochemical changes during growth (Watanabe *et al.* 1983, Dendrinos and Thorpe 1987). The proportion of 20:5*n*-3 and 22:6*n*-3 increases in fed brine shrimp even when the food organisms are deficient in these fatty acids, apparently due to elongation and desaturation of shorter chain *n*-3 fatty acids (Dendrinos and Thorpe 1987).

The levels of *n*-3 HUFA's in brine shrimp can be increased by feeding the nauplii on a lipid emulsion which is rich in these fatty acids (Watanabe *et al.* 1983, Bällaer *et al.* 1985, Sorgeloos *et al.* 1988). The level of *n*-3 HUFA's reaches a maximum level after 12 hours of feeding using this technique (Watanabe *et al.* 1983). However, not all *n*-3 HUFA's are taken up proportionally by this method since brine shrimp incorporate longer fatty acid molecules less readily than shorter chain fatty acids (Dendrinos and Thorpe 1987). The fatty acid composition of brine shrimp can also be enhanced using microencapsulated diets (Sakamoto *et al.* 1982, Walford and Lam 1987). Maximum incorporation of HUFA's by brine shrimp cultured with microcapsules occurs after 8 hours (Walford and Lam 1987).

Other Food Organisms

The freshwater cladocerans *Daphnia* and *Moina* has been used as a supplementary food organism in the intensive rearing of marine fish larvae, including *L. calcarifer*, in South-east Asia (Tattanon and Tiensongrusmee 1984, NICA 1986, Parazo *et al.* 1990, Fermin 1991). *Moina* cultured on yeasts have been found to contain low levels of *n*-3 fatty acids, while those cultured using poultry manure were high in 20:5*n*-3 (Watanabe 1979, Watanabe *et al.* 1983). A major disadvantage in using freshwater cladocerans is that they die rapidly when introduced to sea water and thus small numbers of cladocerans must be added regularly at short intervals (NICA 1986). Alternatively, euryhaline fish larvae, such as *L. calcarifer*, can be transferred to low salinities (c. 10 ppt) for rearing on *Moina* (Parazo *et al.* 1990, Fermin 1991). However, this strategy restricts use of *Moina* as a supplementary food organism to the period when larvae are tolerant of extremely low salinities, i.e. 15 days or older (Fermin 1991).

Copepods have long been recognised as useful supplementary food organisms for the culture of marine fish larvae. The marine copepods *Tigriopus* and *Acartia* contain high levels of both 20:5*n*-3 and 22:6*n*-3 fatty acids, even when cultured on media deficient in HUFA's (Watanabe 1979, Watanabe *et al.* 1983, Witt *et al.* 1984). Better survival and growth rates for larvae fed cultured copepods

compared with larvae fed rotifers and brine shrimp has been demonstrated for mahimahi *Coryphaena hippurus*, mullet *Mugil cephalus* (Kraul 1990) and sobaity *Acanthopagrus cuvieri* (James and Al-Khars 1984). Dhesprasith *et al.* (1986) examined the effects of copepod density on consumption by 14-24 day old larvae of *L. calcarifer*, but supplementary feeding of *L. calcarifer* larvae with copepods does not seem to have been widely adopted.

NUTRITIONAL SUPPLEMENTS FOR MARINE FISH LARVAE

Several nutritional supplements are now available for use in mariculture. There are basically two modes of application of these supplements:

1. Indirect supplementation: this technique involves feeding rotifers and brine shrimp with a supplement which enhances the nutritional composition of the organism prior to feeding to the fish larvae.
2. Direct supplementation: microparticulate or microencapsulated diets with a nutritional composition specifically developed for marine fishes can be fed directly to fish larvae.

There are three main types of supplementary feeds used in intensive larval rearing of marine fishes: liquid, microparticulate and microencapsulated supplements.

A number of liquid enrichment diets have been developed for rotifers and brine shrimp (e.g. 'Selco', produced by Artemia Systems, Ghent, Belgium); the liquid (usually lipid-based) is added to the vessels containing the cultured organisms before they are fed to the fish larvae in order to enhance the nutritional composition of the food organisms. However, these liquid supplements are generally unsuitable for use in tropical aquaculture applications because high water temperatures lead to rapid bacterial decomposition of the supplement with resulting water quality problems in the culture vessels (Rodgers and Barlow 1987).

Microparticulate diets have been developed for use as a substitute for live food organisms in intensive rearing. They have been successfully used to rear a range of temperate marine fish species by wholly or partly replacing rotifers and brine shrimp (Appelbaum 1985, Zitzow and Millard 1988). However, fish fed on microparticulate diets may develop deformities caused by nutritional deficiencies in the composition of the feed (Zitzow and Millard 1988).

Microencapsulated feeds were originally developed as a balanced diet for larval crustaceans. Microcapsules with relatively high levels of HUFA's are now commercially available and the smallest microcapsules (5-20μm) can be used to supplement the nutritional composition of rotifers and brine shrimp fed to fish larvae. Each microcapsule is coated in a protein membrane which prevents

leaching of water soluble nutrients but which is digested when the particle is eaten (Jones *et al.* 1974, Walford and Lam 1987). The membrane coating also delays bacterial decomposition of the microcapsule, allowing for extended immersion of the supplement in the culture vessels without severe degradation of water quality. Frippak Feeds (U.K.) has recently developed a microencapsulated diet specifically for supplementary feeding of live food organisms for marine fish larvae (Frippak 'Booster').

NUTRITIONAL REQUIREMENTS OF BARRAMUNDI LARVAE

The preceding notes indicate that the nutritional deficiencies of live food organisms used in intensive larval rearing of marine fishes are well established. However, the precise nutritional requirements of most marine fish larvae, including barramundi, are not known. In addition, methods of supplementing the nutritional composition of the live food organisms vary considerably. The research described in the following chapters of this report describes experiments which were designed to develop techniques for the nutritional supplementation of barramundi larvae in intensive culture which would increase survival and growth and which could be adopted by commercial hatcheries for minimal cost and with minimal modification to existing procedures and equipment.

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APPENDIX 2.1

FATTY ACID NOMENCLATURE

Fatty acids are described by several systems of nomenclature, although the most common is the shorthand system previously known as the *omega* nomenclature. For example, the fatty acid with the chemical name Eicosatetraenoic acid is also known by its trivial name, Arachidonic acid, and more commonly by its shorthand nomenclature, 20:4*n*-6 (previously 20:4ω6). Less common variations of shorthand nomenclature are 20:4(*n*-6) and 20:4(6).

The shorthand nomenclature indicates the structure of the molecule by describing:

1. the number of carbon atoms in the chain;
2. the number of double bonds;
3. the inclusive number of carbon atoms from the terminal methyl group to the carbon atom of the first double bond.

For example, Arachidonic acid (20:4*n*-6) has 20 carbon atoms and four double bonds with the first double bond located on the 6th carbon atom from the terminal methyl group:

$CH_3 - CH_2 - CH_2 - CH_2 - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH_2 - CH_2 - COOH$

A fatty acid can be elongated by adding further carbon atoms two at a time to the carboxyl end of the molecule, and desturated by forming double bonds between the carboxyl terminal and the nearest double bond to it along the carbon chain. Consequently, the value (*n-x*), where *x* is the number of carbon atoms between the terminal methyl group and the nearest double bond, is fixed. Thus, fatty acids are frequently described as belonging to a particular series, such as the '*n*-3 series' or the '*n*-6 series'.

A fatty acid such 18:3*n*-3 can be elongated and desaturated to form 20:5*n*-3 which in turn can be metabolised to form 22:6*n*-3, but cannot form a fatty acid from another series such as 20:4*n*-6:

18:3*n*-3 (Linolenic acid)

$CH_3 - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - CH_2 - COOH$

20:5*n*-3 (Eicosapentaenoic Acid)
$CH_3 - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH_2 - CH_2 - COOH$

22:6*n*-3 (Docosahexaenoic Acid)
$CH_3 - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH = CH - CH_2 - CH_2 - COOH$

Fatty acids without a double bond are termed saturated fatty acids, those with one double bond are termed monounsaturated, and those with two or more double bonds are termed polyunsaturated (PUFA) or highly unsaturated fatty acids (HUFA).

In addition, the chemical names of fatty acids regarded as essential fatty acids for marine fishes are frequently abbreviated: EPA for eicosapentaenoic acid (20:5*n*-3) and DHA for docosahexaenoic acid (22:6*n*-3). More general references to fatty acids with the same number of carbon atoms, regardless of their degree of saturation, are also abbreviated (e.g. C18 for fatty acids with 18 carbon atoms).

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Chapter 3

EFFECTS OF NUTRITIONAL ENHANCEMENT OF LIVE FOOD ORGANISMS ON GROWTH AND SURVIVAL OF BARRAMUNDI *Lates calcarifer* LARVAE.

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ABSTRACT

Larval and juvenile barramundi *Lates calcarifer* (Bloch) were reared intensively on test diets comprising nutritionally supplemented and unsupplemented rotifers *Brachionus plicatilis* Müller and brine shrimp *Artemia salina* Linnaeus. Both growth and survival of barramundi larvae fed on nutritionally supplemented brine shrimp were superior to that of larvae fed on untreated brine shrimp. Barramundi larvae fed diets incorporating untreated brine shrimp exhibited a mortality syndrome which commenced from 20 to 30 days after hatching and resulted in almost total mortality within the next 10 days. Analyses of the proximate, fatty acid and amino acid composition of the live food organisms used in the test diets, and reference samples comprising barramundi egg yolk and extensively reared juvenile barramundi, suggest that this mortality syndrome was primarily associated with the fatty acid composition of the food organisms, particularly the relative amount of 20:5*n*-3 in the brine shrimp fed to the larvae. These results, and the work of other authors, indicate that there are two mortality syndromes which affect intensively cultured *L. calcarifer* larvae.

INTRODUCTION

Barramundi larvae reared in Australian hatcheries have periodically suffered severe mortalities (up to 90% in some batches) at around 12 to 14 days after hatching. Affected larvae became pale and swam erratically in a corkscrewing motion before dying (MacKinnon 1987). Histological examination of the affected larvae showed extensive vacuolation of the brain and spinal cord and accumulation of excessive fat deposits in the liver (Rodgers and Barlow 1987). The cause of these mortalities has been variously ascribed to nutritional deficiencies in the live food organisms fed to the barramundi larvae (Rodgers and Barlow 1987) and to the action of a picorna-like virus in the larvae (Glazebrook *et al.* 1990, Glazebrook and Heasman 1992, Munday *et al.* 1992). Similar symptoms have been described for a mortality syndrome seen in *L. calcarifer* larvae reared in Tahiti (AQUACOP *et al.* 1990, Renault *et al.* 1991).

This syndrome has not recurred at the barramundi hatchery at the Northern Fisheries Centre (NFC), Cairns, since supplementary feeding techniques for rotifers and brine shrimp were adopted in 1987, using a microencapsulated diet (Frippak 'CAR 1' and 'Booster') known to contain high levels of HUFA's (Walford and Lam 1987). This supports the hypothesis that the syndrome may have been caused by nutritional deficiencies in the diet of the larvae (Rimmer *et al.* 1988).

Another mortality syndrome has been described for *L. calcarifer* larvae reared in hatcheries in the Philippines (Dhert *et al.* 1990). Mortalities commenced 23 days after hatching and this syndrome was found to be associated with deficiencies of HUFA's in the brine shrimp component of the larval diet (Dhert *et al.* 1990).

In order to investigate the cause of the observed mortality syndrome in intensively reared barramundi and to develop larval diets which would provide maximal survival and growth, a series of experiments was conducted on the nutritional requirements of barramundi larvae. The experiments described in this chapter were designed to:

1. determine whether the mortality syndrome observed in larval barramundi 12-14 days after hatching could be ascribed to nutritional deficiencies in the live food organisms used to rear barramundi intensively;
2. investigate the nutritional influences which affect survival and growth in intensively reared barramundi;
3. increase the survival of barramundi reared intensively by developing techniques to enhance the nutritional composition of the live food organisms used in intensive rearing.

MATERIALS AND METHODS

Barramundi larvae used in these experiments were reared from fertilised eggs obtained from wild-spawning barramundi at Weipa in the north-eastern Gulf of Carpentaria in northern Queensland. The fertilised eggs were air-freighted to Cairns and arrived around the time of hatching (12-17 h after fertilisation at 28-30°C).

For convenience, the term 'larva' has been used to describe the fish used in these experiments, although the larger fish used in these experiments are properly termed 'juvenile' (Kendall *et al.* 1984). Barramundi larvae metamorphose at 8-12 mm in length (MacKinnon 1987, Russell 1987). Similarly, the term 'nauplius' is used to include the various naupliar stages of brine shrimp.

Experimental Larval Rearing Unit

The experimental larval rearing unit used in these experiments comprised 22 individual chambers each of 2 l capacity (Fig. 3.1a). Water from a header tank was gravity-fed to the rearing chambers, from which it drained via small nylon screens (60, 120 or 200μm aperture) allowing water exchange while retaining the food organisms (Fig. 3.1b). The water from the rearing chambers collected in a sump and was pumped back to the header tank via a biological filter (Figs 3.1 & 3.2). Temperature was maintained at 29$\pm$1°C by air-conditioning the room and heating water in the header tank. Lighting was provided by fluorescent lamps at an intensity of 400 lux at the water surface. Water quality parameters (temperature, pH, salinity, ammonia, nitrite and nitrate) were monitored daily.

The larvae were fed twice daily to ensure that they had constant access to freshly supplemented food organisms. Before each feed, approximately 90% of the water from each chamber was siphoned through a large surface area screen (200 or 400μm) to remove the remaining food organisms while retaining barramundi larvae.

Larval Feeding Schedule

Rotifers were reared outdoors on the microalga *Nannochloropsis oculata* (CSIRO isolate CS-179) together with small quantities of yeast. Rotifers were harvested daily in the morning and fed to the barramundi larvae that afternoon and the following morning. Rotifers used in the afternoon were supplemented with a microencapsulated diet (Frippak 'Booster') in aerated 3 l bottles for 4 h at 1.0 g microcapsule dry weight (DW) / 4 $\times$ 10^6 rotifers / l. Those used the next morning were supplemented for 20-22 h at 0.2 g microcapsule DW / 2 $\times$ 10^6

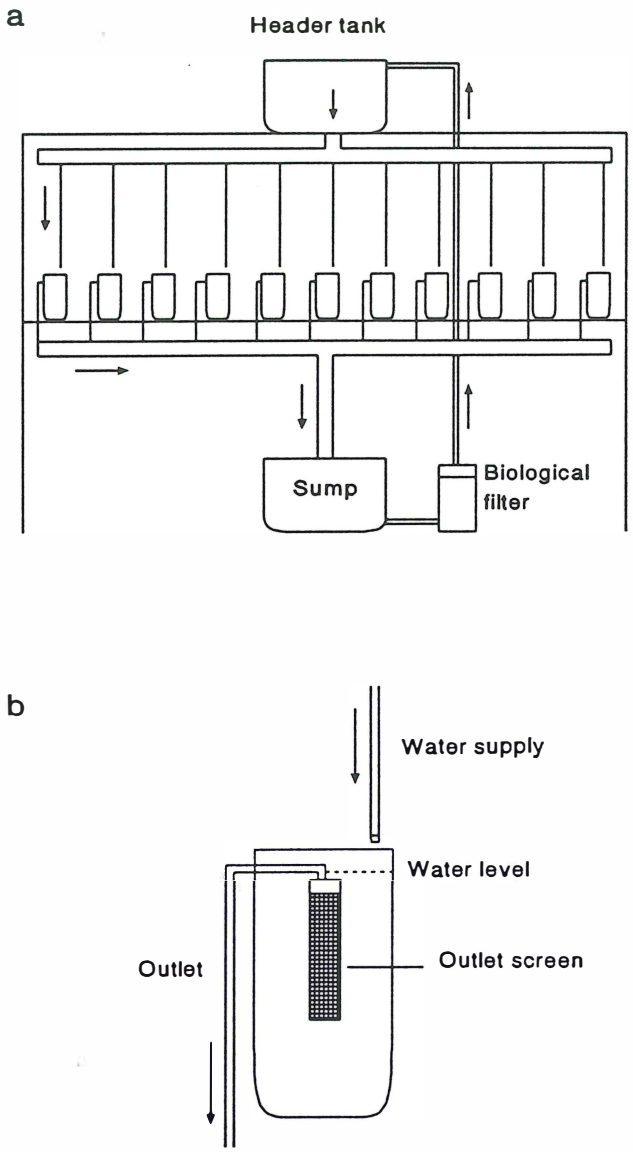

Figure 3.1. Diagram of the experimental larval rearing unit used for the nutrition experiments described in this chapter: (a) general arrangement of experimental unit, and (b) details of larval rearing chamber. Arrows indicate the direction of water flow within the unit. (Note diagrams not to scale).

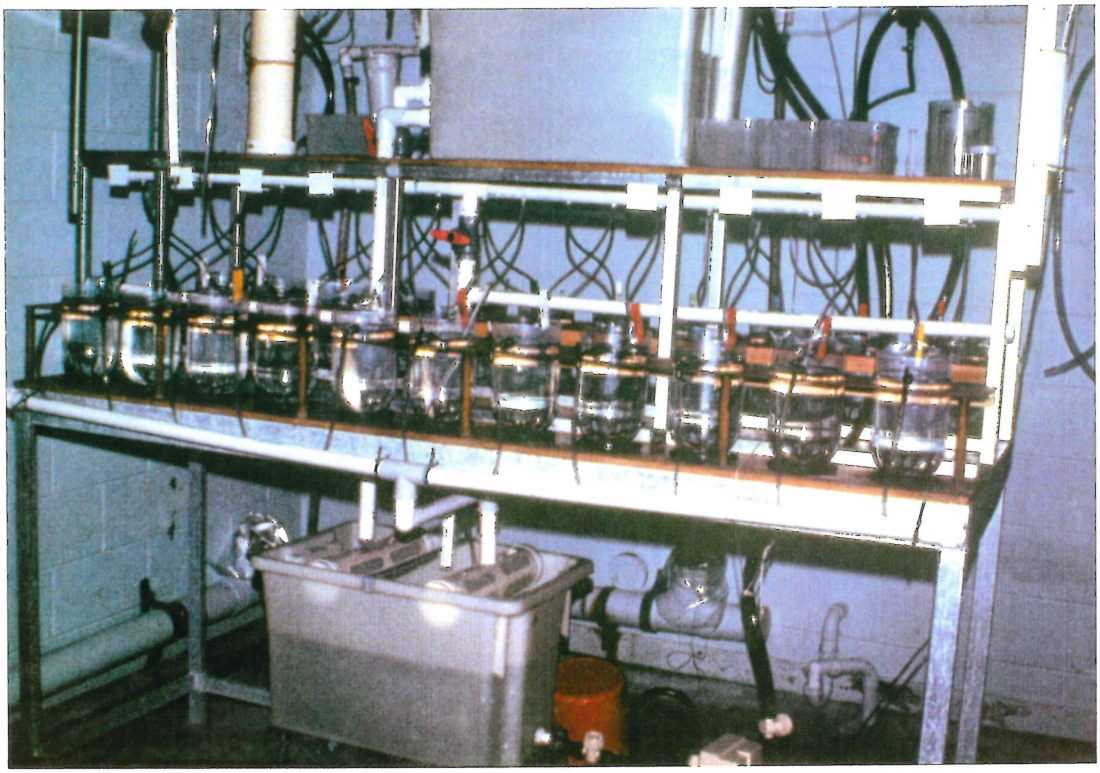

Figure 3.2. Photograph of experimental larval rearing unit used for nutrition experiments with barramundi larvae.

rotifers / l. Rotifers not supplemented with microcapsules were retained in aerated 3 l bottles at the same density as supplemented rotifers.

Brine shrimp nauplii ('Aquarium Products' brand; origin Columbia) were harvested daily and fed immediately ('freshly hatched' treatment) or starved for 24 h to ensure that the yolk was absorbed before they were offered to the barramundi larvae ('starved' treatment) or were supplemented. Supplementary feeding of brine shrimp took place in a 50 l glass aquarium or a 70 l hemispherical fibreglass tank. Microcapsules (Frippak 'Booster') were added at 0.3 g microcapsule DW / 0.5-1.0 $\times$ 10^6 nauplii/l.

Barramundi larvae were fed rotifers at 10-20 rotifers/ml from day 2 (where hatching is designated as day 1) to day 14, and brine shrimp at 2 nauplii/ml from day 8, increasing to 5 nauplii/ml by day 12 and continuing at this density until the completion of each experiment.

Reference samples were taken from two sources to allow a comparison of natural dietary regimes with those applied in the hatchery. Dendrinos and Thorpe (1987) suggested that the biochemical composition of the egg yolk reflects the nutritional requirements of the larvae because the egg yolk is the sole source of nutrition prior to the commencement of exogenous feeding. Oocytes were stripped from running-ripe female barramundi caught at Weipa and the chorion removed by filtering the macerated egg material through 20μm mesh. The filtered egg yolk was then used for biochemical analyses.

Juvenile barramundi reared in a brackishwater earthen pond were also used for reference samples. These fish were reared on naturally occurring zooplankton (Rutledge and Rimmer 1991) and can thus be assumed to have a biochemical composition similar to juvenile barramundi in the wild which recruit to similar habitats in estuarine mangrove swamps (Russell and Garrett 1985).

Experimental Design

The experimental larval rearing unit was used to test 4 replicates of 4 diets (experiments N1 and N2) or 3 diets (N3) in a block design. The rotifer and brine shrimp treatments used for the experimental diets in these three experiments are listed in Table 3.1. Experiment N1 was used to determine the effect of the test diets on survival, whereas N2 was used to examine the effects of the same diets on growth. Experiment N3 was used to further differentiate the effects of different brine shrimp treatments on survival of barramundi larvae. The same procedures and experimental conditions were used in all experiments.

Larvae used in each experiment were taken from a single 1200 l tank containing hatched larvae. Because direct counting of larvae resulted in severe but highly variable mortality, three 50 ml samples from the tank containing hatched larvae were counted to provide an estimate of larval density, and a volume of water estimated to contain approximately 500 larvae was added to each rearing container. Five additional volumes were preserved for enumeration after the larvae had been introduced to the rearing chambers and these indicated that approximately 550 larvae were introduced to each chamber for experiment N1 and 400 larvae for N3. Low numbers of barramundi larvae available for experiment N2 resulted in only about 130 larvae being added to each rearing container for this experiment.

Mortalities could only be accurately estimated after about day 10, when larvae were large enough to leave visible corpses. Experiment N1 was terminated at day 30 when cumulative mortalities in two treatments were at or near 100%. Experiment N2 was terminated at day 22, when all survivors were preserved in

10% formalin for later measurement of total length (TL); this measurement was used to compare growth between treatments. Experiment N3 was terminated at day 40 when cumulative mortalities in two treatments approached 100%.

Key to abbreviations in Tables 3.1-3.5.

Rotifer Treatments

CR: rotifers reared on *N. oculata*;

SR: rotifers reared on *N. oculata*, then supplemented with microcapsules;

Brine Shrimp Treatments

FH: freshly hatched brine shrimp;

St: brine shrimp starved for about 24 h after hatching to allow yolk resorption;

Supp: brine shrimp harvested about 24 h after hatching, then supplemented with microcapsules.

Reference

Eggs: egg yolk component of barramundi oocytes stripped from running-ripe female fish at Weipa;

Fish: whole juvenile barramundi reared in an earthen pond containing naturally occurring zooplankton.

Table 3.1.

Treatments of rotifers and brine shrimp used for the test diets in experiments N1-N3. See text for key to abbreviations.

Experiment	N1, N2	N3
Diet 1	CR, FH	SR, FH
Diet 2	SR, FH	SR, St
Diet 3	CR, St/Supp*	SR, Supp
Diet 4	SR, St/Supp*	-

* Larvae fed starved brine shrimp (St) from day 8 to day 12, then supplemented brine shrimp (Supp) from day 13.

Statistical Analyses

Analysis of variance (ANOVA) and Tukey's HSD multiple range test were carried out on TL data from experiment N2 using the statistical package StatGraphics. Patterns in mortality over time were compared by fitting proportional hazards models with the statistical package GLIM. This approach is

widely used in medical research and relates the probability of failure for a treatment to some baseline risk (or hazard) by estimating a quantity termed the hazard ratio. Although the baseline hazard may vary with time, the hazard ratio for any treatment is assumed to remain constant. The methods used are similar to those described by Bartlett (1978).

Histology

Samples of dead and live moribund larvae were taken for histological examination at irregular intervals. Most of the larvae sampled live at day 22 in experiment N2 (a total of 389 larvae) were used for histological examination. Specimens for histology were preserved in 10% formalin, and forwarded to the Queensland Department of Primary Industries' (QDPI) veterinary laboratory at Oonoonba where the specimens were processed using conventional wax embedding techniques, sectioned and stained with haematoxylin and eosin (J. Norton, pers. comm.).

Biochemical Analyses

Four to six replicate samples of each dietary treatment (incorporating organisms from both morning and afternoon feeds), ten samples of barramundi oocytes and two samples of barramundi juveniles were used for the biochemical analyses. Samples of rotifers and brine shrimp were sieved to remove small particles such as algal cells and uneaten microcapsules before preparation for analysis.

Proximate Composition

The samples were weighed and freeze-dried for storage prior to analysis. Aliquots were taken from a prepared homogenised sample for the following analyses. Moisture was determined in duplicate by drying 2g samples to constant weight at 100°C. Fat was extracted using the method of Folch *et al.* (1957) and determined by evaporating a portion of the chloroform (fat) layer from the extraction and weighing the residue. Protein content was determined using the Kjeldahl method for total nitrogen and applying a factor of 6.25 to calculate crude protein. Ash was determined by drying 4g samples on a steam bath for 2h, igniting at 550°C in a furnace overnight then weighing the cooled residue. Carbohydrate content was calculated by difference.

Fatty Acid Composition

The samples were extracted with chloroform/methanol (2:1 v/v) using the modified methods of Folch *et al.* (1957) and Bligh and Dyer (1959). The samples were stored under nitrogen at -25°C until analysed. The excess solvent was removed using a rotary evaporator and the lipid residue taken up in a minimum of hexane. The base-catalysed transesterification procedure of Christopherson and Glass (1969) was used to prepare the fatty acid methyl esters from the lipid solution. The esters were separated by gas-liquid chromatography on a Shimadzu R1-A with a 2.1m x 3mm i.d. glass column packed with 15% OV-275 on 100/120 Chromosorb PAW-DMCS. The column oven was temperature programmed from 190° to 220°C increasing at 2°C/min and the carrier gas (nitrogen) flow rate was 65 ml/min.

The peaks were identified and quantified on a Shimadzu RPR-G1 GC processor calibrated using the methyl esters of authentic triacyloglycerol standards supplied by Sigma (Sigma Chemical Co., St Louis, MO, USA). A comparison was also made with a standard methylated cod liver oil sample supplied by R. Johns of the University of Melbourne.

Amino Acid Composition

The samples were weighed and freeze dried for storage prior to analysis, then further dried in a vacuum desiccator over P_2O_5. The dry samples were fat extracted with hexane, ground in an agate pestle and mortar, and placed in bottles in a vacuum desiccator over P_2O_5. 0.5g of each sample were taken for Kjeldahl Nitrogen analysis. These results were corrected for fat content and expressed as g/kg crude protein (N x 6.25) on sample DM basis. 200mg of each sample was hydrolysed with 20ml 6N HCl in a sealed tube under nitrogen for 18 hours at 110°C for amino acid analysis. 100mg of each sample was treated with performic acid prior to hydrolysis for the estimation of cystine and methionine. Samples were analysed for amino acids using ion exchange chromatography in a Waters amino acid analyser.

RESULTS

Water Quality

Water temperature in the experimental unit ranged from 28-30°C; pH from 7.8-8.0; salinity from 27-32 g/l; total ammonia from 0-0.2 mg/l; nitrite from 0-0.2 mg/l; nitrate was constant at about 20 mg/l.

Survival

Experiments N1 and N2

The four test diets used in experiments N1 and N2 showed dramatically different effects on survival of barramundi larvae. Patterns of mortality were similar in both N1 and N2, despite the different initial densities used in N2. Larvae fed diets 1 and 2 began showing stress symptoms (pale colouration, erratic swimming followed by 'fainting') on day 18. Large-scale mortalities began on day 20 (N1) or day 21 (N2), and continued until most larvae were dead within the next 5 or 6 days (Fig. 3.3). In comparison, larvae fed diets 3 and 4 had substantially less mortalities over the same period. Diet 2 (which incorporated supplemented rotifers) showed consistently higher mortality over the duration of the experiment than diet 1 (which incorporated unsupplemented rotifers); and, similarly, diet 4 showed consistently higher mortality than diet 3 (Fig. 3.3).

Analysis of the mortality data from experiment N1 used the unsupplemented diet 1 as the baseline treatment. Supplementation of brine shrimp substantially reduced the hazard (i.e. increased the probability of survival) for diets 3 and 4 relative to diets 1 and 2 (Table 3.2). In comparison, supplementation of rotifers slightly increased the hazard for diets 2 and 4 relative to diets 1 and 3. No interaction between the effects of supplementing rotifers and brine shrimp was found. The lowest hazard ratio was associated with diet 3 (Table 3.2) indicating that this was the diet which produced maximal survival in barramundi larvae over the duration of the experiment.

Table 3.2.

Hazard ratios for test diets using supplemented and unsupplemented rotifers and brine shrimp (diets 1 - 4) from experiment N1.

		Rotifers	
		CR	SR
Brine shrimp	FH	1.000 (Diet 1)	1.785 (Diet 2)
	St/Supp	0.014 (Diet 3)	0.026 (Diet 4)

Experiment N3

The three brine shrimp diets used in experiment N3 also showed substantially different effects on survival of barramundi larvae. Extensive mortalities of barramundi larvae began on day 19 (diet 1) and day 27 (diet 2) and continued

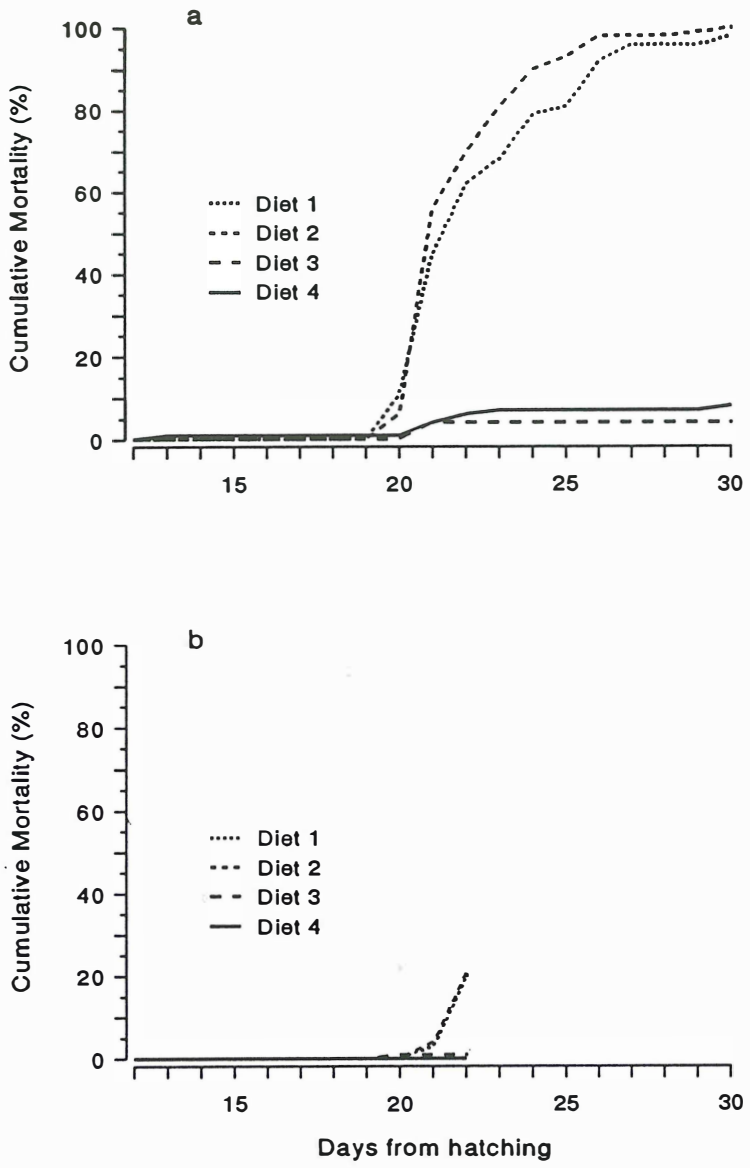

Figure 3.3. Cumulative mortality of barramundi larvae fed 4 test diets in experiments (a) N1 and (b) N2. Plotted values represent means of four replicates.

Treatments (see text for details):

Diet 1	CR, FH
Diet 2	SR, FH
Diet 3	CR, St/Supp
Diet 4	SR, St/Supp

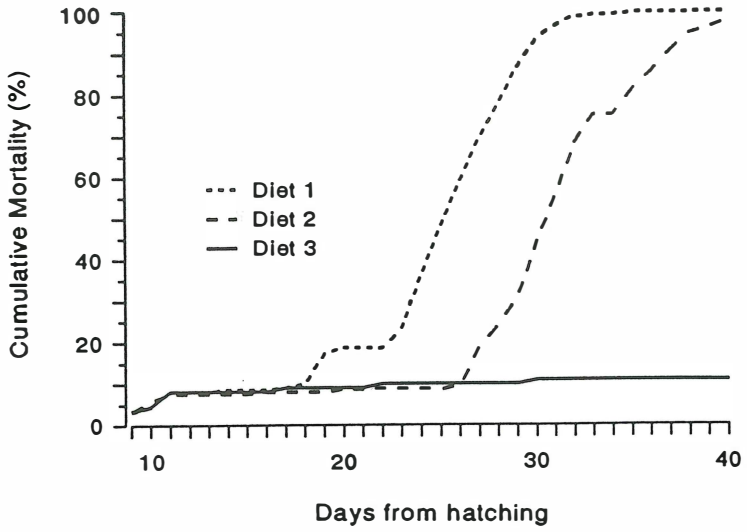

Figure 3.4. Cumulative mortality of barramundi larvae fed 3 test diets in experiment N3. Plotted values represent means of four replicates.
Treatments (see text for details):
Diet 1 SR, FH
Diet 2 SR, St
Diet 3 SR, Supp

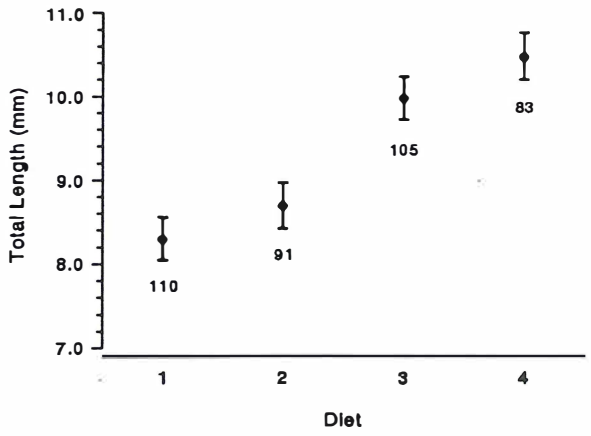

Figure 3.5. Total length of barramundi larvae fed on four text diets in experiment N2 and measured at day 22. Means and 95% confidence limits shown; numbers below bars represent sample sizes.
Treatments (see text for details):
Diet 1 CR, FH
Diet 2 SR, FH
Diet 3 CR, St/Supp
Diet 4 SR, St/Supp

until most of the fish in these treatments were dead by day 40 (Fig. 3.4). Larvae fed supplemented brine shrimp (diet 3) showed only negligible mortalities over the same period (Fig. 3.4). The hazard ratio for diet 1 relative to diet 2 in experiment N3 was 0.21, indicating that barramundi larvae fed starved brine shrimp exhibited a higher probability of survival than those fed freshly hatched brine shrimp.

Growth

The four test diets used in experiments N1 and N2 produced significantly different growth rates in barramundi larvae by day 22 after hatching (ANOVA, $F=59.5$, $P<0.01$). Larvae fed on diets 3 and 4 were significantly larger at day 22 than those fed on diets 1 and 2 (Tukey's HSD, $P<0.05$). Larvae fed on diets 1 and 2 averaged 8.29 mm TL and 8.69 mm TL respectively at day 22, while larvae fed on diets 3 and 4 averaged 9.98 mm TL and 10.48 mm TL respectively at day 22 (Fig. 3.5).

The effects of rotifer and brine shrimp supplementation on growth were further analysed using TL data from experiment N2 and rotifer supplementation, brine shrimp supplementation and experimental block as factors. The results of this analysis indicated that barramundi larvae fed on supplemented rotifers (in diets 2 and 4) were significantly larger at day 22 than those fed on unsupplemented rotifers (diets 1 and 3) (ANOVA, $F=13.7$, $P<0.01$). Larvae fed on unsupplemented rotifers averaged 9.11 mm TL while those fed on supplemented rotifers averaged 9.54 mm TL at day 22 (Fig. 3.6a).

Similarly, barramundi larvae fed on starved and supplemented brine shrimp (diets 3 and 4) were significantly larger at day 22 than those fed on newly hatched brine shrimp (diets 1 and 2) (ANOVA, $F=158.9$, $P<0.01$). Larvae fed on newly hatched brine shrimp averaged 8.47 mm TL while those fed on starved and supplemented brine shrimp averaged 10.20 mm TL at day 22 (Fig. 3.6b).

Histology

Larvae fed on diets incorporating starved or supplemented brine shrimp showed no abnormal pathology. Several larvae fed on diets incorporating freshly hatched brine shrimp showed some minor vacuolation of the spinal cord at day 22. The extensive vacuolation of the brain and spinal cord seen in larvae reared in previous seasons was not observed in any larvae reared in these experiments.

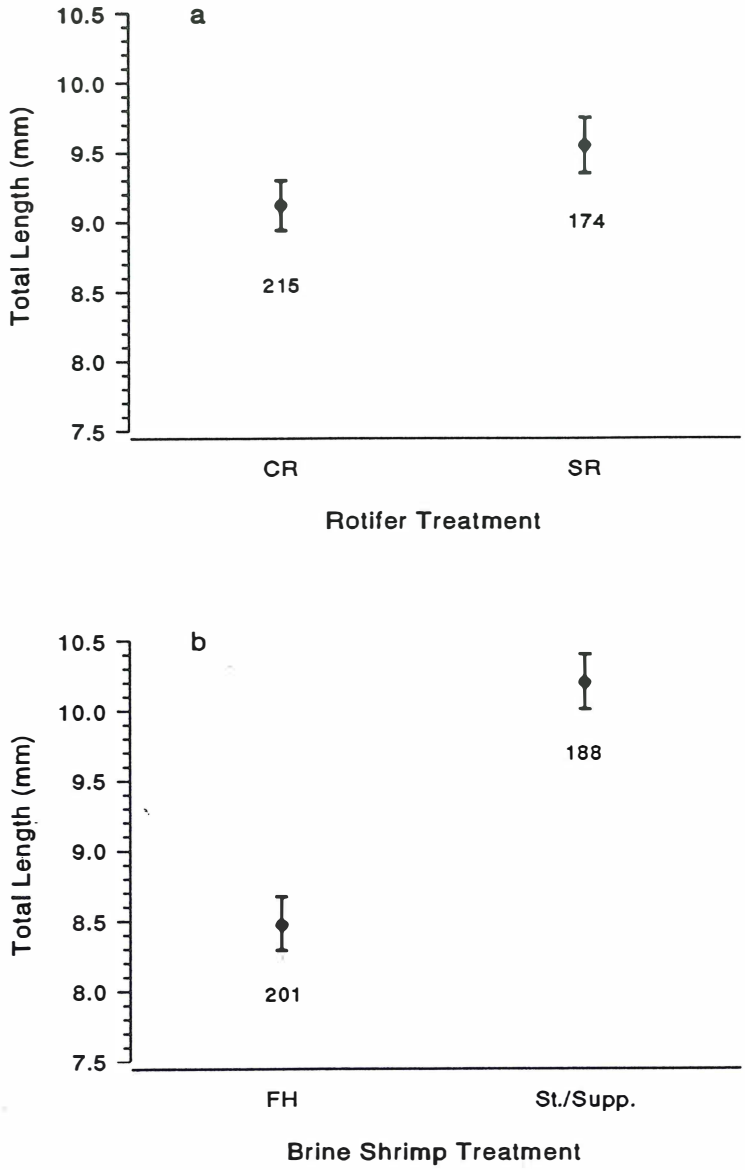

Figure 3.6. Total length of barramundi larvae fed on four test diets in experiment N2 and measured at day 22.
(a) Larvae fed on unsupplemented rotifers (diets 1 and 3) and supplemented rotifers (diets 2 and 4).
(b) Larvae fed on freshly hatched brine shrimp (diets 1 and 2) and starved and supplemented brine shrimp (diets 3 and 4).
Means and 95% confidence limits shown; numbers below bars represent sample sizes.

Biochemical Composition

Since only minor differences in the biochemical composition were found between replicate samples of the dietary treatments, barramundi oocytes and pond-reared barramundi juveniles, data from replicate samples for each treatment and reference were averaged.

Proximate Composition

Only minor differences were found between the proximate composition of the live food organisms used in the test diets and the reference samples (Table 3.3). Eggs and juvenile barramundi had higher levels of fat and moisture, and lower levels of carbohydrate and ash, compared with the live food organisms fed to the barramundi larvae. Supplementation of brine shrimp resulted in a decrease in the relative amount of carbohydrate and concomitant increases in ash.

Fatty Acid Composition

The fatty acid composition of the rotifers more closely matched the fatty acid composition of juvenile barramundi than that of the barramundi egg yolk (Table 3.4). Unsupplemented rotifers were deficient in 4 fatty acids found in barramundi egg yolk: 18:4, 22:4*n*-6, 22:5*n*-6 and 22:6*n*-3. Supplementation of the rotifers incorporated 22:6*n*-3 into the diet and resulted in minor changes in the proportions of other unsaturated fatty acids. Both unsupplemented and supplemented rotifers were deficient only in 18:4 in comparison with barramundi juveniles.

Freshly hatched and starved brine shrimp were deficient in 6 fatty acids found in barramundi egg yolk: 15:0, 20:4*n*-6, 22:4*n*-6, 22:5*n*-3, 22:5*n*-6 and 22:6*n*-3. Supplemented brine shrimp were deficient in only 3 fatty acids in comparison with barramundi egg yolk: 20:4*n*-6, 22:4*n*-6 and 22:5*n*-6. Supplementation of brine shrimp resulted in levels of 20:5*n*-3 increasing from about 1% to 8%, and incorporation of 15:0, 22:5*n*-3 and 22:6*n*-3 into the diet.

Freshly hatched and starved brine shrimp were deficient in 3 fatty acids found in barramundi juveniles: 15:0, 20:4*n*-6, 22:5*n*-3. Supplemented brine shrimp were deficient only in 20:4*n*-6 in comparison with barramundi juveniles.

There were substantial differences in the fatty acid composition of barramundi egg yolk and barramundi juveniles. Juvenile barramundi lacked 3 fatty acids found in the egg yolk: 22:4*n*-6, 22:5*n*-6 and 22:6*n*-3. Barramundi egg yolk contained much higher levels of 22:6*n*-3 than was found in any of the dietary treatments.

Amino Acid Composition

No substantial differences were found in the amino acid composition of the live food organisms and the reference samples used in these experiments (Table 3.5). Supplementation of rotifers and brine shrimp resulted in only minor changes in the amino acid composition of these organisms.

Table 3.3.

Proximate composition of live food organisms used in experiments N1-N3, barramundi egg yolk and pond-reared juvenile barramundi. See text for key to abbreviations.

Composition (%)	Rotifer		Brine shrimp			Reference	
	CR	SR	FH	St	Supp	Eggs	Fish
Protein	51.3	52.2	49.4	50.2	50.5	50.1	54.2
Fat	7.7	9.4	16.4	14.3	14.4	20.0	18.1
Carbohydrate	15.2	14.2	16.6	12.9	9.3	8.0	7.7
Ash	18.2	16.9	9.6	13.1	16.9	5.3	8.7
Moisture	7.6	7.3	8.0	9.5	8.9	16.6	11.3

Table 3.4.

Fatty acid composition of live food organisms used in experiments N1-N3, barramundi egg yolk and pond-reared juvenile barramundi. See text for key to abbreviations.

% Total Fatty Acids	Rotifer		Brine shrimp			Reference	
	CR	SR	FH	St	Supp	Eggs	Fish
14:0	2.7	3.6	0.5	0.5	0.7	1.3	8.3
15:0	1.0	1.0	-	-	0.2	1.1	1.1
16:0	10.4	12.3	10.8	10.8	9.4	26.5	18.3
16:1	22.2	19.0	5.9	5.6	6.1	8.6	22.1
18:0	3.2	5.8	6.1	6.3	6.7	8.3	8.3
18:1	27.8	22.3	22.0	22.1	20.9	17.6	15.5
18:2	6.3	8.9	10.8	9.9	12.4	4.6	5.4
18:3/20:1	6.6	5.0	23.1	23.0	15.8	1.5	2.0
18:4	-	-	13.0	11.6	9.6	2.6	2.8
20:4*n*-3	0.5	1.0	2.5	2.3	2.1	-	-
20:4*n*-6	2.7	2.3	-	-	-	3.9	1.5
20:5*n*-3	11.5	13.0	0.9	2.1	7.7	4.4	7.4
22:1	2.2	1.1	6.2	6.2	6.8	-	-
22:4*n*-6	-	-	-	-	-	1.3	-
22:5*n*-3	3.3	2.5	-	-	0.7	3.4	1.8
22:5*n*-6	-	-	-	-	-	1.2	-
22:6*n*-3	-	2.5	-	-	1.4	17.2	-

Table 3.5.

Amino acid composition of live food organisms used in experiments N1-N3, barramundi egg yolk and pond-reared juvenile barramundi. See text for key to abbreviations.

Amino Acids (g/16gN DM)	Rotifer		Brine shrimp			Reference	
	CR	SR	FH	St	Supp	Eggs	Fish
Lysine	5.9	6.1	6.3	6.2	7.0	7.8	7.7
Histidine	1.3	1.4	1.5	1.6	1.6	2.6	1.9
Arginine	5.3	5.6	7.0	7.0	6.4	6.8	7.1
Aspartic acid	7.8	8.0	7.6	7.9	8.1	7.7	11.1
Threonine	3.4	3.5	4.0	4.1	4.1	4.8	4.1
Serine	4.2	4.4	5.0	4.8	4.9	5.9	3.9
Glutamic acid	10.1	10.7	11.0	11.1	10.9	12.7	13.3
Proline	4.1	4.0	4.4	4.1	4.3	4.6	3.6
Glycine	3.2	3.3	4.1	4.2	4.2	3.3	6.3
Alanine	3.5	3.8	4.6	4.9	5.5	8.4	6.1
Valine	4.5	4.6	4.8	4.8	4.9	6.9	4.6
Methionine	1.2	1.3	1.9	1.7	1.7	2.2	2.7
Isoleucine	4.3	4.3	4.5	4.5	4.6	6.5	4.0
Leucine	6.8	7.0	6.6	6.7	7.1	9.7	7.2
Tyrosine	3.0	3.2	3.3	3.4	3.8	4.4	3.0
Phenylalanine	3.8	4.0	3.7	3.8	3.8	4.6	3.7
Cystine	1.6	1.6	1.3	1.4	1.3	1.1	1.0

DISCUSSION

The mortality syndrome previously observed in larval barramundi at day 12-14 was not seen during these experiments and could not be induced using rearing techniques identical to those used in previous seasons (experiment N1, Diet 1) when this mortality syndrome caused substantial mortalities in some batches of larvae (MacKinnon 1987, Rodgers and Barlow 1987). Thus the results of these experiments do not support the hypothesis that this syndrome is primarily related to nutritional deficiencies in the live food organisms used for intensive rearing of barramundi larvae (MacKinnon 1987, Rodgers and Barlow 1987, Rimmer *et al.* 1988).

A picorna-like virus has been found in *L. calcarifer* larvae affected by this syndrome in hatcheries in Australia (Glazebrook *et al.* 1990, Glazebrook and Heasman 1992, Munday *et al.* 1992) and in Tahiti (Renault *et al.* 1991), although a causal link has not yet been established which would indicate that this virus is a primary pathogen of fish larvae (Glazebrook and Heasman 1992).

The mortality syndrome observed in the barramundi larvae used in these experiments appears to be identical to that described by Dhert *et al.* (1990) in *L. calcarifer* larvae reared in the Philippines and ascribed to a deficiency in the levels of *n*-3 HUFA's in the brine shrimp fed to the fish larvae. The results of the experiments presented in this paper support this conclusion, as most of the variation in the nutritional composition of the live food organisms used for intensive larval rearing of barramundi was found in the fatty acid composition, with relatively little variation in the proximate and amino acid composition of the test diets and reference samples.

A comparison of the test diets using supplemented rotifers (diets 2 and 4) in experiment N1 indicated that barramundi larvae fed these diets exhibited slightly lower survival but slightly higher growth rates than those fed diets containing unsupplemented rotifers (diets 1 and 3). Since unsupplemented rotifers were deficient in 22:6*n*-3 (Table 3.4) and supplementation resulted in the incorporation of this fatty acid in the diet, these results indicate that the presence of 22:6*n*-3 in the diet of barramundi larvae during the rotifer feeding stage had no positive effect on survival, although it may have contributed to growth.

In most marine fishes, including barramundi, the egg yolk is high in 22:6*n*-3 which is quickly reduced during larval development, suggesting that the larval requirement for 22:6*n*-3 is initially met from this source and an exogenous source is not required during this stage (Watanabe *et al.* 1989, Ostrowski and Divakaran 1990, Webster and Lovell 1990). Marine fish larvae generally are incapable of elongating and desaturating shorter-chain fatty acids to produce

22:6*n*-3 (Witt *et al.* 1984, Watanabe *et al.* 1989, Ostrowski and Divakaran 1990, Webster and Lovell 1990) and are thus dependent on endogenous stores or dietary sources of 22:6*n*-3. The larvae of dolphin fish (*Coryphaena hippurus*) are subject to substantial mortality following the exhaustion of endogenous levels of 22:6*n*-3 if an alternative source is not provided (Ostrowski and Divakaran 1990). Similarly, barramundi larvae may have their initial requirement for 22:6*n*-3 met by the high levels of this fatty acid in the yolk (Table 3.4) and may not require an exogenous source of 22:6*n*-3 until this is exhausted. However, the absence of 22:6*n*-3 in pond-reared juveniles (Table 3.4) suggests that this fatty acid is not conserved by barramundi larvae. Thus the importance of 22:6*n*-3 in the diet of barramundi larvae remains uncertain.

The results of experiment N1 also indicate that the effects of supplementation of the rotifer component of the diet are relatively small, whereas supplementation of brine shrimp has marked effects on growth and survival of barramundi larvae. Experiment N3 provided further information on the effect of different brine shrimp treatments on survival of barramundi larvae.

Diets 1 and 2 in experiment N3 both caused extensive mortality of barramundi larvae, but barramundi larvae fed diet 2 began dying 8 days after larvae fed diet 1 and continued to die at about the same rate, while diet 3 was the only diet adequate for long-term larval survival (Fig. 3.4). This pattern suggests that some nutritional factor was present at a very low level in diet 1 and at a higher, but still inadequate, level in diet 2 and was only present at an adequate level in diet 3. This is further supported by the proportional hazards analysis of these results which indicated that the probability of failure associated with diet 2 was significantly lower than that of diet 1 (hazard ratio 0.21).

The concentration of 20:5*n*-3 in the three brine shrimp treatments is the only measured biochemical component of the diets that reflects this pattern. Starvation of brine shrimp for about 24 h after hatching increased the levels of 20:5*n*-3 from about 1% to 2%, while supplementation of brine shrimp increased the levels of this fatty acid to about 8%. Supplementation of brine shrimp with microcapsules resulted in the incorporation of 15:0, 22:5*n*-3 and 22:6*n*-3 into the diet (Table 3.4), but the addition of these fatty acids in diet 3 does not readily explain the observed differences in mortality between diets 1 and 2. The levels of 20:5*n*-3 in supplemented brine shrimp are similar to those found in pond-reared barramundi (Table 3.4).

The confounding effects of the different levels of various fatty acids found in the treatments used in these experiments prevent a detailed analysis of the precise roles of individual fatty acids in promoting growth and survival of barramundi larvae. Further research is required to investigate the role of fatty acids from

endogenous and exogenous sources to determine precisely the biochemical requirements of barramundi larvae during and after absorption of the yolk and oil globule.

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Chapter 4

EVALUATION OF MICROPARTICULATE DIETS FOR REARING BARRAMUNDI *Lates calcarifer* LARVAE

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ABSTRACT

Attempts to partly or completely replace live prey organisms used for intensive rearing of barramundi with a prepared microparticulate diet (Zeigler 350 AP), were unsuccessful. Barramundi larvae fed on the microparticulate diet alone and a combination of microparticulate diet and brine shrimp exhibited lower survival and lower growth rates than barramundi larvae fed on brine shrimp alone. Factors which may have affected the poor acceptance of this inert diet by barramundi larvae are: availability of the microparticulate diet and perception of the microparticles by the larvae.

INTRODUCTION

The production of live prey organisms is one of the most expensive aspects of intensive larval rearing because of the facilities and staff required for production of algae, rotifers and brine shrimp. In order to reduce production costs, there have been attempts to replace live food organisms, partly or completely, with inert diets. In addition to their economic advantages, inert diets can be prepared to the precise nutritional requirements of the species being cultured and hence overcome many of the nutritional deficiencies associated with live prey organisms (Person-Le Ruyet 1990). Additional components, such as feeding attractants, can be also incorporated into inert diets (Person-Le Ruyet 1990).

Although several freshwater fish species have been reared exclusively on microparticulate or microencapsulated diets, notably carp, *Cyprinus* spp., and whitefish, *Coregonus* spp., (Dabrowski *et al.* 1984, Rösch and Appelbaum 1985, Zitzow and Millard 1988), attempts to rear marine fish larvae using such diets exclusively have generally resulted in poor survival (Adron *et al.* 1974, Person-Le Ruyet 1990). However, partial replacement of live prey organisms by microparticulate or microencapsulated diets has generally been more successful than complete replacement for marine fish larvae, although larvae reared on a combined diet of live prey and inert diet generally show inferior growth and survival compared with those fed only on live prey (Kanazawa *et al.* 1982, 1989, Appelbaum 1985, Person-Le Ruyet 1990, Walford *et al.* 1991).

The experiment described in this paper was designed to investigate the feasibility of using a commercially available microparticulate diet to partly or completely replace brine shrimp during intensive larval rearing of barramundi.

MATERIALS AND METHODS

Barramundi larvae used in this experiment (designated experiment M1) were reared from fertilised eggs obtained from broodstock held at N.F.C. and spawned using hormone induction techniques (Garrett and Connell 1991). The larvae were fed on supplemented rotifers and supplemented brine shrimp according to the schedule described previously. Following the completion of the rotifer feeding stage (day 15), approximately 50 barramundi larvae were transferred to each of 12 containers in the experimental unit described previously.

This experiment tested 4 replicates of 3 diets in a block design. The test diets consisted of brine shrimp and microparticles introduced to the containers at a

total density of 5/ml. This density was based on the density of brine shrimp used for intensive larval rearing of barramundi. The diets tested were:

Diet 1: brine shrimp at 5 nauplii/ml;
Diet 2: brine shrimp at 2.5 nauplii/ml and microparticulate diet at 2.5 microparticles/ml;
Diet 3: microparticulate diet at 5 microparticles/ml.

Brine shrimp and pellets were introduced to the experimental containers twice daily. Brine shrimp densities were determined volumetrically, as described previously. The microparticulate diet used in this experiment was Zeigler 350 AP (i.e. Artificial Plankton with a nominal particle diameter of 350μm). To determine the number of microparticles per unit weight, 20 samples of Zeigler 350 AP were weighed and the number of microparticles counted, giving an estimated 83,000 microparticles per gram. At each feed, 0.06 g and 0.12 g of Zeigler 350 AP was added to the containers with barramundi larvae fed diet 2 and diet 3 respectively, to provide the required numbers of microparticles to achieve the densities listed above.

Mortalities were monitored daily by counting dead larvae. Experiment M1 was terminated at day 24 and all surviving larvae were preserved in 10% formalin. TL data from these larvae were used to compare growth between treatments with ANOVA and Tukey's HSD test using the statistical package StatGraphics.

RESULTS

This experiment revealed various problems with the use of microparticulate diets in a standard intensive larval rearing system. Circulation in the containers was inadequate to suspend the microparticles in the water column and the microparticles sank rapidly to the bottom of the containers. The barramundi did not appear to feed on microparticles which had settled to the bottom of the tank.

Survival

The three test diets used in this experiment resulted in markedly different survival during the experiment. Barramundi larvae fed a diet of microparticles alone (diet 3) began dying in large numbers on day 21 and substantial mortalities continued to occur up until the end of the experiment at day 24 (Fig. 4.1a). The combination of microparticles and brine shrimp (diet 2) resulted in a lower mortality during the experiment, and the diet comprising brine shrimp alone (diet 1) resulted in only negligible mortalities over the same period (Fig. 4.1a).

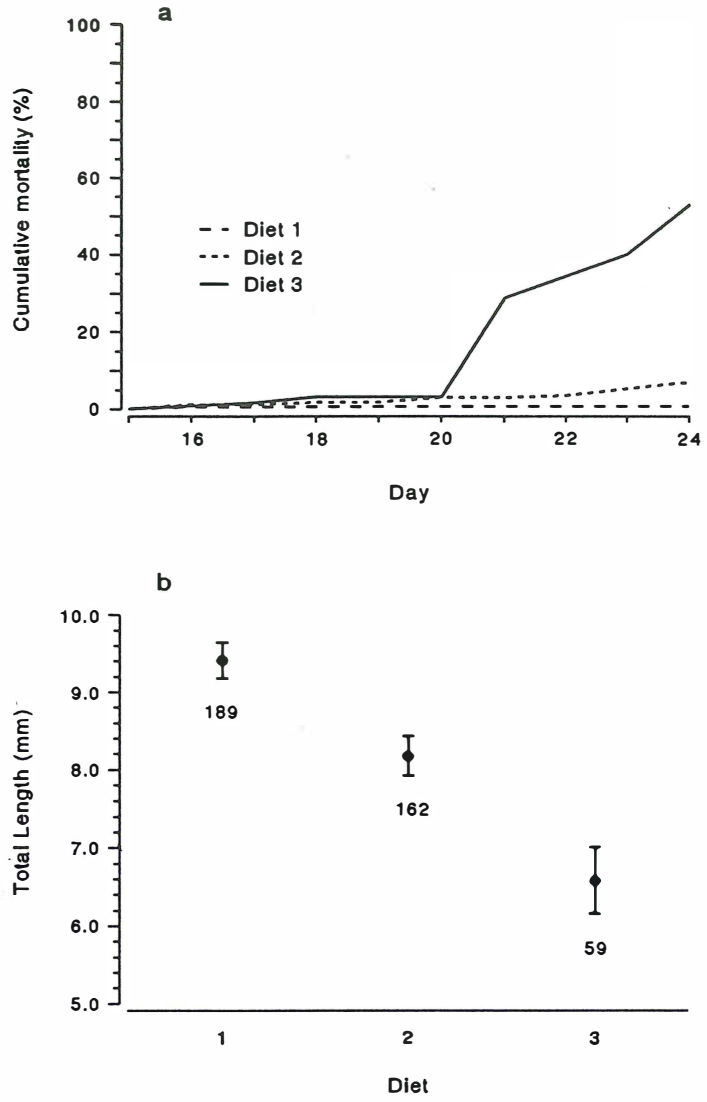

Figure 4.1. Survival and growth of barramundi larvae in experiment M1:
(a) Cumulative mortality of barramundi larvae fed three test diets in experiment M1. Plotted values represent means of four replicates.
(b) Total length of barramundi larvae fed three test diets in experiment M1 and measured at day 24. Means and 95% confidence limits shown; numbers below bars represent sample sizes.
Treatments (see text for details):
Diet 1 Brine shrimp only;
Diet 2 Brine shrimp and microparticulate diet;
Diet 3 Microparticulate diet only.

Growth

Surviving barramundi larvae fed the three diets used in experiment M1 were significantly different in length at day 24 (ANOVA, $F=71.8$, $P<0.01$; Tukey's HSD, $P<0.01$). Block effects and interaction terms were not significant (ANOVA, $P>0.05$). Larvae fed diets 1, 2 and 3 averaged 9.4, 8.2 and 6.6 mm TL respectively at day 24 (Fig. 4.1b).

DISCUSSION

The results of this experiment indicate that inert diets cannot be readily substituted for brine shrimp in the intensive larval rearing of barramundi. Although the use of brine shrimp and the microparticulate diet in combination produced better survival and growth than the microparticulate diet alone, this appeared to be due to the brine shrimp component of the diet rather than any effect of the microparticulate diet. Observation of the experimental animals during the course of this experiment indicated that acceptance of the inert diet was very poor. Some of the factors which may have contributed to this poor acceptance are: availability of the inert diet and perception of the inert diet by the larvae.

Availability of the diet was effectively low because the particles sank rapidly to the bottom of the container. This condition did not simulate the behaviour of brine shrimp which remained in suspension due to their active swimming. Since suspension of particles depends on the degree of aeration and the size and shape of the tank (Backhurst *et al.* 1989), a major redesign of the tanks used for larval rearing of barramundi would be necessary to accommodate the use of microparticulate diets.

Perception of the microparticles may have been limited by factors such as their colour (and hence their contrast against the background of the containers). Growth and survival of barramundi larvae are strongly influenced by the visibility of prey items (Pearce 1991) and the microparticles may be of insufficient contrast with the tank background to be perceived by the larvae.

Many marine fish larvae require live prey in combination with inert diets to achieve satisfactory growth and survival (Kanazawa *et al.* 1982, 1989, Walford *et al.* 1991). Walford *et al.* (1991) found that *L. calcarifer* larvae fed a microencapsulated diet alone died by day 10 after hatching, whereas those fed a combination of rotifers and microcapsules for 5 days, then microcapsules alone had a survival rate of 2.4%. The movement of live prey may be necessary to

stimulate feeding by the larvae (Appelbaum 1985, Person-Le Ruyet 1990), particularly in the case of predatory fish such as barramundi. The use of live prey in combination with microcapsules may also be necessary to aid digestion in fish larvae, since the live prey may also serve as an important source of digestive enzymes and bacteria in larvae whose digestive systems have not yet fully developed (Appelbaum 1985, Walford *et al.* 1991).

The age at which larvae are introduced to inert diets also affects the successful use of such diets. Several species have been successfully reared from first feed on inert diets (Adron *et al.* 1974, Dabrowski *et al.* 1984, Rösch and Appelbaum 1985, Zitzow and Millard 1988), while in other species inert diets could only be used to replace brine shrimp (Dabrowski *et al.* 1984, Appelbaum 1985). Microparticulate diets were not used in this study to replace rotifers in the early larval rearing of barramundi because of the poor quality of smaller particles. A high proportion of such particles were made up from individual components of the original preparation, and thus could not be presented as a homogeneous diet. However, the poor survival recorded by Walford *et al.* (1991), i.e. 2.4%, using rotifers and a microencapsulated diet to rear *L. calcarifer* larvae, suggests that substitution of inert diets during the rotifer feeding stage of barramundi larval rearing has similar problems to those encountered in this experiment.

Overall, the use of inert diets to partly or completely replace live food organisms in the intensive larval rearing of barramundi shows little potential and the development of techniques to allow the reliable use of inert diets would require considerable further research. Among the factors which need to be addressed are: redesign of tanks to allow prolonged suspension of microparticles, the development of microparticles of lower specific gravity which remain suspended in the water column for longer, research into the factors affecting perception of the microparticles by barramundi larvae, and the nutritional composition of the inert diet. The generally poor survival of barramundi larvae reared on inert diets, even under optimal conditions (Walford *et al.* 1991), suggest that this technique has limited commercial application since any cost savings made by decreasing the reliance on live food organisms may be offset by decreased larval survival.

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Chapter 5

CULTURE OF COPEPODS FOR MARINE FISH LARVAL REARING

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ABSTRACT

The literature pertaining to culture techniques for copepods was reviewed to determine the most promising approach to developing large-scale techniques for copepod culture in hatcheries. Extensive techniques, which use only fertilisers and light as inputs, and avoid the need for costly monoaxenic microalgal cultures, were found to be suitable for the culture of copepods. Experimental cultures were maintained, with partial harvesting, for 74 days. These techniques, when scaled up for hatchery use, can be used to provide copepods as a HUFA-rich dietary item for marine fish larvae.

INTRODUCTION

The intensive culture of marine fishes requires a source of zooplanktonic food organisms. To date, this requirement has been largely met by the development of culture techniques for rotifers (*Brachionus plicatilis*) and brine shrimp (*Artemia salina*) for use in intensive hatcheries. However, the nutritional composition of both rotifers and brine shrimp may be inadequate, particularly in terms of the relative amounts of highly unsaturated fatty acids (HUFA's) they contain. One response to this problem has been the development of techniques to enhance the nutritional composition of rotifers and brine shrimp using supplements high in HUFA's or other required nutrients.

An alternative approach to the problem is the culture of food organisms with a nutritional composition more closely suited to the nutritional requirements of the cultured species. Zooplanktonic crustaceans, particularly copepods and cladocerans, are important components of the diet of marine fish larvae in the wild and have been shown to be particularly high in the HUFA's required by most marine fish larvae (Watanabe 1979, Watanabe *et al.* 1983, Witt *et al.* 1984). Better survival and growth rates for larvae fed cultured copepods compared with larvae fed rotifers and brine shrimp have been demonstrated for turbot *Scophthalmus maximus* (Nellen *et al.* 1981), mahimahi *Coryphaena hippurus*, mullet *Mugil cephalus* (Kraul 1990) and sobaity *Acanthopagrus cuvieri* (James and Al-Khars 1984). This is due not only to the better HUFA profile of copepods, but also their higher calorific value compared with rotifers (Theilacker and Kimball 1984). Dhesprasith *et al.* (1986) examined the effects of copepod density on consumption by 14-24 day old larvae of *L. calcarifer*, but supplementary feeding of *L. calcarifer* larvae with copepods does not seem to have been widely adopted.

The overall aim of the work described in this section was to evaluate the potential of less intensive culture techniques for copepods and to establish whether such techniques are suitable for the routine culture of copepods in hatcheries for supplementary feeding of barramundi larvae. Large-scale culture techniques for copepods are not widely established. Consequently, this component of the research program commenced with a review of existing published information on copepod culture techniques to determine research directions for developing practical procedures that could be readily adopted by marine finfish hatcheries with minimal additional technology and facilities. The information obtained in the review was used to undertake several trials in developing basic culture techniques for tropical marine copepods.

REVIEW OF CULTURE TECHNIQUES

A number of representatives of each of three Orders of the Sub-Class Copepoda (Harpacticoida, Calanoida and Cyclopoida) are recorded as having been cultured with varying degrees of success. Generally, harpacticoid copepods have been cultured with more success than either calanoid or cyclopoid copepods (Kahan *et al.* 1982).

Uhlig (1984) listed the suitability of copepods as live prey organisms for mariculture as:

1. their tolerance of a wide range of environmental conditions;
2. their ability to utilise different food sources;
3. their high reproductive capacity;
4. their relatively short life cycle;
5. their ability to produce high population densities in appropriate culture systems.

These topics are discussed in greater detail below.

Environmental Conditions

Environmental conditions such as temperature, salinity and light may influence the survival, reproduction, and metabolic activities of copepods in culture (Nassogne 1970).

Temperature: Ambient temperature has been generally used for copepod culture. Zurlini *et al.* (1978) cultured the marine harpacticoid *Euterpina acutifrons* at 18°C because this temperature represented that of the natural habitat from which the initial culture was obtained. Most other culture experiments have been undertaken at temperatures between 21 and 33°C (Kahan *et al.* 1982, Ohno and Okamura 1988, Ohno *et al.* 1990). James and Al-Khars (1984) found that best survival of the cyclopoid *Apocyclops borneoensis* was obtained at 27-30°C.

Salinity: Copepods from fresh, brackish and salt water have been successfully cultured. Most harpacticoids have higher and wider salinity tolerances than other copepods. James and Al-Khars (1984) found that although survival of the cyclopoid *Apocyclops borneoensis* was highest at a salinity of 20 ppt, this species could be adapted to and reared in salinities of 30-40 ppt.

Lighting and Photoperiod: Most indoor culture has been undertaken under 12:12 light:dark regime, although Szyper (1989) chose continuous fluorescent light. Kraul (1990) suggested partial shading from direct sunlight for outdoor cultures. Rieper's (1984) experiments on selective feeding of harpacticoids included on

uninterrupted 12 hour dark cycle to enable the copepods to settle and feed without disturbance.

Culture Vessels: Culture vessels used for large-scale copepod culture generally range from $15x10^3$ to $24x10^3$ litres. Uhlig (1984) indicated that the mass production of *Tisbe* is essentially related to the available substrate area and less to available water volume.

Food Sources

Copepods must be provided with food suitable in quantity and quality that allows them not only merely to survive, but to develop and reproduce (Nassogne 1970). Copepods utilise two methods of feeding: filtration of smaller particles, and handling of larger cells that may only be partially ingested (Nassogne 1970). The particular technique varies with the size of cell captured, but this adaptability allows copepods to utilise a wide range of food sources. A variety of food types have been used in attempts to culture copepods, including: unicellular microalgae, yeasts, bacterial cultures, minced mussel flesh, and lettuce leaves (which decay and promote blooms of bacteria and protozoans).

Most workers have adopted the techniques traditionally used to culture rotifers and brine shrimp for copepod culture. Attempts to culture copepods using monoaxenic algal cultures have met with varied success. The nutritional value of different algal species may vary widely, and some algal species do not allow reproduction at all while other species do not allow development to adulthood (Nassogne 1970). Algal cultures with mixed species composition, or which contain bacteria or micronutrients have been shown to support more generations, and to support egg production rates 2-20 times higher, than monoaxenic cultures, presumably due to a better nutritional profile from mixed cultures (Nassogne 1970, Haney 1973, Kinne 1977).

The marine calanoid *Acartia tsuensis* has been cultured by using poultry manure as a basal fertiliser and adding ammonium sulphate, urea, superphosphate of lime and 'Clewat' (major ingredient EDTA) to the culture medium (Ohno and Okamura 1988, Ohno *et al.* 1990). Maximal exploitation rates were obtained when the chlorophyll *a* concentration was maintained at about 10μg/litre. Zurlini *et al.* (1978) found that $2x10^5$ cells/litre was a sufficient concentration of phytoplankton to maximise the values of the weight and compositional parameters of *E. acutifrons*.

Life Cycle

Zurlini *et al.* (1978) detailed times for developmental stages in the life cycle of *E. acutifrons*. The newly hatched nauplius I took 5-7 days to reach copepodite I stage. Males reached the adult stage at 9-11 days after the appearance of nauplius I, while for females this period was 10-12 days. Estimated life spans for copepods vary substantially, and are particularly dependent on the temperature of the culture and the composition of the algal food source used. For example, the estimated life span of *Euterpina acutifrons* ranges from 8-11 days for culture at 24-26°C (Kraul 1990), to 14.3-38.3 days for the same species at 18°C (Nassogne 1970). The variation in the latter estimate is attributed to the use of different algal species as the food source (Nassogne 1970).

The body size of copepods is influenced by water temperature and food abundance, but the observed increase in the body size of females at low population densities may be an adaptation to increase fecundity, since female body size and fecundity are positively correlated (Ohno and Okamura 1988, Ohno *et al.* 1990).

Recruitment is inhibited by high densities of adult copepods, either through cannibalism of nauplii or through limited food availability, but recruitment increases at low population densities (Ohno and Okamura 1988, Ohno *et al.* 1990). The productivity and egg production rate of *A. tsuensis* cultures increased with increasing chlorophyll *a* concentrations up to 10μg/litre (Ohno and Okamura 1988). The inhibitory effects observed at high population densities of *Tisbe* spp. were overcome by regular water exchange in the cultures (Kahan *et al.* 1982).

Density and Harvest

Maximum densities obtained in intensive culture were between $1x10^4$ and $1x10^5$ copepods/litre. Several researchers have investigated the maximum harvest that copepod cultures could sustain but the results were variable. Ohno *et al.* (1990) maintained *A. tsuensis* cultures at exploitation rates of 27-30% per day, while James and Al-Khars (1984) exploited only 4% of the cultures' maximum density. Nellen *et al.* (1981) harvested 10% (volume) per day of culture tanks in which the calanoid copepod *Eurytemora* predominated, and found that this exploitation rate resulted in a 'stabilised density' of around 1-2 nauplii/ml. Kahan *et al.* (1982) harvested 1-2 kg of *T. japonicus* each day for 2 weeks from 50-200 tonne capacity tanks with densities of 2-5 copepods/ml.

Anaesthetising copepods with dilute MS222 (0.06% MS222 in seawater) relaxed the appendages and permitted more effective sieving. Anaesthetised animals were quickly revived in seawater (Theilacker and Kimball 1984).

Conclusions

The results summarised above indicate that copepods are not particularly amenable to culture using the intensive techniques which are commonly used for rotifers and other live food organisms. The success of culture techniques which use a variety of algal species, as well as other food sources such as protozoa and bacteria, indicate that an extensive style of culture is more applicable to the large scale culture of copepods. Extensive culture involves the production of live food organism in a system where the major inputs are fertilisers and light to enhance primary productivity. A secondary benefit of extensive culture techniques is that it is relatively inexpensive, because the labour and equipment costs associated with production of monoaxenic algal cultures are not required. Accordingly, the approach taken to developing culture techniques for copepods as part of the present study was to investigate extensive culture techniques.

MATERIALS AND METHODS

Extensive Culture Trial

An inoculant of 500 litres of water from a brackishwater larval rearing pond containing naturally occurring zooplankton, including rotifers and copepods (Rutledge and Rimmer 1991), was added to a 1200 litre fibreglass tank. The tank was topped up with sea water filtered to 1μm and the salinity adjusted to that of the pond water (28 ppt).

Inorganic and organic fertilisers (diammonium phosphate [DAP] and lucerne pellets respectively), were initially added to the tank at 1 g and 100 g per 1000 litres of water respectively. Throughout the duration of the experiment, inorganic and organic fertiliser were added in response to a decline in the density or condition (as measured by the number of eggs carried) of the rotifer population, or a decline in the proportion of gravid female copepods.

The culture tank was located in an exposed position. Salinity was maintained at 25-30 ppt for most of the experiment, although rainfall caused the salinity to drop to 2.2 ppt on one occasion.

Culture Techniques Trial

From the original extensive culture, six 2000 litre flat bottomed tanks (diameter 2.15 m., height 0.6 m.) were inoculated with approximately $6x10^5$ copepods each. Three combinations of inorganic and organic fertiliser were used to test whether any particular fertiliser regime was superior for copepod culture (Table 5.1). Sterilised water, as used in one treatment, was treated with chlorine at 10 ppm residual chlorine and then dechlorinated using sodium thiosulphate at 0.07 g/l.

Table 5.1. Water source and fertilisers used for copepod culture techniques trial.

Tanks	Water source	Fertiliser
1 & 2	sterilised water	200 g lucerne and 2 g DAP
3 & 4	unsterilised water	200 g lucerne and 2 g DAP
5 & 6	unsterilised water	horse manure and 2 g DAP

These tanks were located under cover to prevent drastic salinity changes during periods of heavy rainfall. During the experiment, salinity in these tanks ranged from 26.2 to 30.8 ppt and temperature ranged from 16 to 25°C.

Estimation of Copepod Density

One litre samples were collected from each tank and filtered through a 62μm (nominal mesh width) screen. Zooplankters retained on the screen were preserved in 10% formol-acetic alcohol (FAA). Subsamples (1 ml) of each sample were taken and the number of copepods was counted in a revolving circular plastic tray under a binocular microscope.

RESULTS

To clean the copepod culture tanks, the airlines were removed, the tank swirled and left to settle, then the debris drained from the bottom. The tank was topped up with seawater, the salinity was adjusted, and fertiliser was added as necessary. It was found that this method of cleaning resulted in large numbers of copepods being discarded, presumably because they were feeding on the bacteria present among the debris.

The tanks which contained sterilised water (tanks 1 and 2) exhibited a much greater increase in copepod density than the other two treatments (tanks 3-6), and after one month tanks 3-6 were discarded. Green filamentous algae grew on the bottom and sides of the tanks, and then peeled off and floated to the surface. These algal filaments contained numerous harpacticoid copepods and several

techniques were developed to allow adult copepods, copepodites and nauplii to be harvested from the algae.

Copepodites and adult copepods could be physically washed out of the algal filaments by agitating the algal mass over a 120μm mesh screen. To harvest the nauplii, a 120μm mesh screen containing masses of filamentous algae was placed over a 62μm mesh harvester, and water was pumped through it overnight using air lifts. As the copepod nauplii hatched, they dropped through the 120μm screen into the harvester, and were collected and counted the following day.

These experimental cultures were maintained for 74 days (Fig. 5.1). Copepod densities fluctuated drastically over the course of this trial (Fig. 5.1). Copepod density in both tanks increased steadily until the tanks were harvested, which resulted in a drastic decline in copepod density (Fig. 5.1). Copepod densities again increased after each harvest.

DISCUSSION

This research has shown that copepods can be readily cultured using extensive techniques where additions of organic and inorganic fertilisers promote increased primary productivity in the culture vessel. This primary production is directly utilised as a food source by the copepods. Because extensive culture techniques support a diverse range of algae, bacteria and protozoa, copepods can utilise a range of food types, thus ensuring adequate nutrition to support growth and reproduction (Nassogne 1970). The culture techniques used in this research were applied on a relatively small scale because of equipment and funding limitations and because of a greater emphasis on the development of the nutritional supplementation techniques described earlier. However, previous experience with extensive aquaculture techniques indicates that these culture techniques can readily be scaled up for hatcheries where larger quantities of copepods are required.

The suitability of copepods as a food source for larval barramundi has been shown in other experiments (Rutledge and Rimmer 1991). The benefit of copepods as a food source in intensive fish hatcheries is either as a supplementary food item which is high in HUFA's, or as a replacement for the traditional food items in such hatcheries: rotifers and brine shrimp. Recent research has shown that rotifers and brine shrimp may be nutritionally inadequate food items for many marine fish species, and this has provoked renewed interest in the development of techniques to culture alternative food organisms such as copepods. The present study, while necessarily limited in extent, has shown that copepods can be cultured relatively easily using facilities and techniques

which are already found in intensive fish hatcheries, without creating an additional demand on algal culture or other resources.

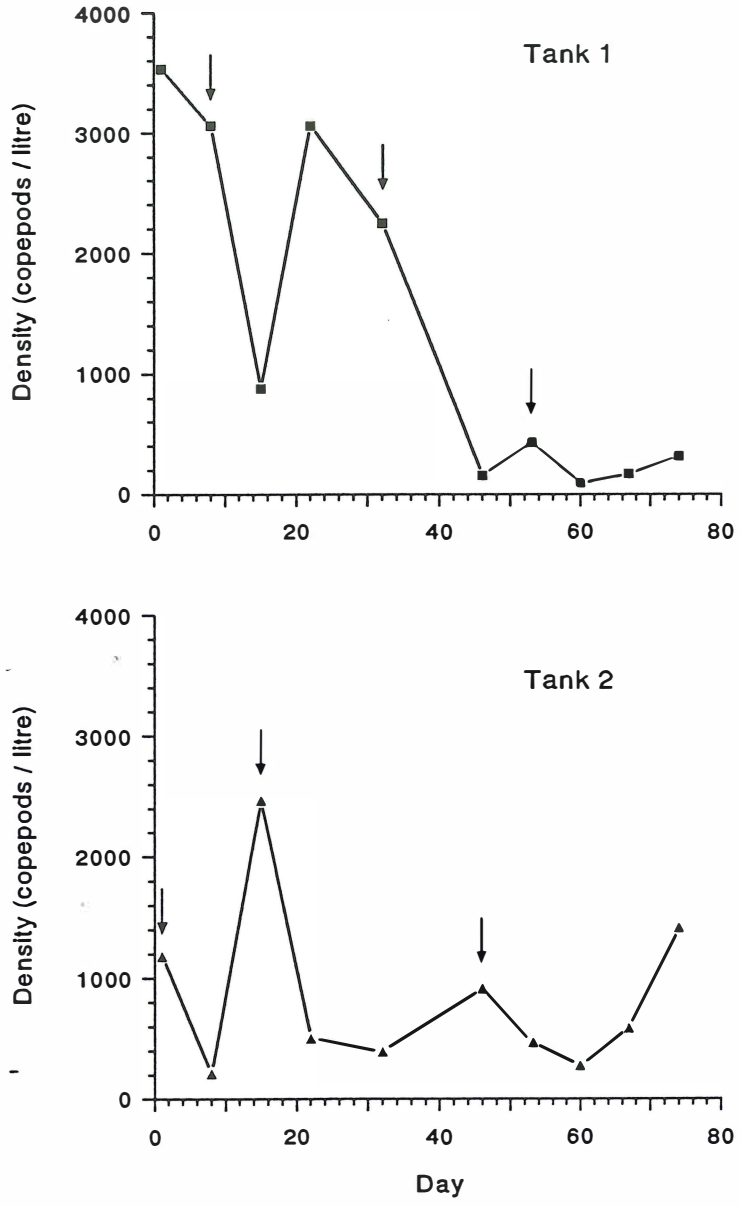

Figure 5.1. Density of copepods in tanks 1 and 2. Arrows indicate occasions when nauplii and copepods were harvested from the tanks.

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Chapter 6

OPTIMAL SIZE FOR WEANING BARRAMUNDI *Lates calcarifer* FRY ONTO ARTIFICIAL DIETS

Authors: C.G. Barlow and L.J. Rodgers

ABSTRACT

Literature reports have indicated that weaning barramundi *Lates calcarifer* fry from live zooplankton onto formulated dry diets is often undertaken with fry as small as 10 mm total length (TL). We conducted an experiment to determine if survival during weaning was affected by the size of the fry at the initiation of weaning. Four trials were undertaken using fry initially 12.8, 13.6, 16.7 and 19.6 mm mean TL. Each trial was replicated four times with 200 fish per replicate. At the outset of the trials, feeding of live zooplankton was discontinued and a commercially available salmon starter crumble was dispensed by automatic feeders every hour for the 12 hours of daylight (photoperiod 12L/12D). Survival through the 10-day weaning period averaged 39, 58, 97 and 92% for the 12.8, 13.6, 16.7 and 19.6 mm fry respectively. An asymptotic curve described the relationship between initial size and survival, and indicated that survival of greater than 90% can be expected with fry greater than 16 mm TL at the time of weaning. This size accords with that at which barramundi fry change their feeding habit from that of a zooplanktivore to a predator. Hatchery managers need to determine the optimum size for weaning barramundi fry based on the costs involved in intensive rearing using live foods and the improved survival with increasing size up to 16 mm TL at the initiation of weaning.

INTRODUCTION

The transition from live food to artificial diets is an important period in the rearing of fish larvae or fry. In general terms, the transition must be accomplished as early as possible, because of the costs involved in live food production, while at the same time not incurring excessive mortalities due to the fish being too small to adapt to the artificial food. In this context, failure to adapt can be due to either a physiological problem (the food is nutritionally unsuitable) or a physical problem (the food is rejected because of particle size, taste or texture).

Barramundi or sea bass (*Lates calcarifer*) is a catadromous fish which is farmed throughout southeast Asia and in tropical Australia (Copland and Grey 1987). Literature reports have indicated that weaning onto inert foods (formulated dry diets, or minced fish, prawns or meat) is generally commenced when barramundi are about 20 days old, or 10 mm total length (TL) (Awang 1987; MacKinnon 1987; Maneewong 1987; Tucker et al. 1988). At this length, barramundi have not completed metamorphosis (scales are not apparent until the fish are 11 mm TL). None of these reports have indicated the degree of mortality associated with weaning barramundi at this size.

Studies by Barlow *et al.* (1993) have shown that barramundi undergo an abrupt change in feeding habit at about 16-18 mm TL. Less than 17 mm TL, the fish are roving zooplanktivores. Larger than 17 mm TL, they change rapidly to a lurking predator mode of feeding, eating small insect larvae in addition to zooplankton. The change in diet is associated with changes in pigmentation and feeding behaviour. We hypothesised that survival of fry through the weaning period would be enhanced if weaning is commenced once the fish have adopted the lurking predator mode of feeding. Consequently, we conducted an experiment to determine the survival and ease of training barramundi fry onto a formulated dry diet at various sizes between 12 and 20 mm TL.

MATERIALS AND METHODS

Fish and facilities

The fish were obtained from hormone-induced spawnings of captive broodstock. The larvae were reared in salt water until approximately 10 mm TL, then transferred to fresh water and maintained in 1500 l tanks at 26-30°C. Prior to the commencement of each trial, the fish were fed *Artemia* nauplii and zooplankton harvested from freshwater ponds.

The experiment was conducted indoors in steeply-sided, conical-based tanks, 800

mm deep and 130 l capacity (Fig. 6.1). Water was exchanged at the rate of 4 l/min via a central stand pipe which was screened to prevent the escape of fish. Temperature was maintained at 28.0±0.5°C. Air was supplied via an air-stone in the bottom of each tank. Photoperiod was 12L/12D. Artificial feed was delivered by automatic feeders positioned over each tank.

Experimental procedure

The experimental design consisted of 4 treatments replicated 4 times. The treatments were fish of different initial TLs (mean±s.d.), as follows (CV = coefficient of variation);

T1: 12.8±0.94 mm, n = 53, CV = 7.3%
T2: 13.6±1.36 mm, n = 71, CV = 10.0%
T3: 16.7±1.42 mm, n = 50, CV = 8.5%
T4: 19.6±1.42 mm, n = 60, CV = 7.3%

Because of a shortage of facilities, it was not possible to test the four treatments simultaneously on the same batch of fish. Thus, four trials (corresponding to the four treatments) were conducted over a two month period, using fish from different spawnings.

At the start of each trial, 200 healthy fish were placed into each of the four replicate tanks and maintained on zooplankton for one day. Any mortalities during this period, assumed to be due to handling, were replaced. Thereafter, feeding of zooplankton was stopped and the dry diet (Skretting 0.3-0.6 mm salmon starter diet) was dispensed every hour for 12 hours (darkness one hour after the last feed). At each feeding, 5 g of food was dispensed, which was in excess of the requirements of all sized fish. The tanks were cleaned each morning by scrubbing the walls, turning off the air and water and siphoning out the settled debris. Dead fish were collected and counted. The trials were conducted for 10 days, which was sufficient time for all surviving fish to be fully adapted to the dry diet. The fish were counted and measured when the trials were terminated. The difference between the number of missing fish at the end of the trial and the number of dead fish recorded during the trial was assumed to be due to cannibalism.

Analysis of data

Survival data were analysed by analysis of variance (arcsin transformation of percentage data) and non-linear regression analysis.

RESULTS

The percentage survival, TL at the termination of the trial, and the percentage of fish which died due to starvation and cannibalism are listed in Table 6.1. Analysis of variance of the percentage survival data indicated that there was a significant difference ($P<0.001$) in survival between the treatments. Least significant difference tests showed that there were three groups in which the mean survival was not significantly different ($P<0.05$), namely T4 and T3, T3 and T2, and T2 and T1. The relationship between TL and percentage survival was described by the equation

$$y = 96.85 - 155981(0.540^{l}) \qquad (R^2 = 0.88, P<0.01)$$

where y = percentage survival and 1 = TL (Figure 8.2).

Survival within treatments was uniform, with the exception of the fourth replicate in T2, wherein the survival was 79% compared with 46%, 51% and 54.5% for the other replicates (Figure 6.2). There was no obvious explanation for the higher survival in one replicate.

Table 6.1.

Survival and mortalities due to starvation and cannibalism (expressed as percentages) of barramundi fry of various sizes during 10-day weaning trials. The data are means ± standard deviation.

Treatment	Initial TL (mm)	Final TL (mm)	% Survival	% Mortalities due to	
				Starvation	Cannibalism
T1	12.8 ± 0.9	20.1 ± 3.0	38.9 ± 5.8	59.8 ± 5.4	2.8 ± 2.6
T2	13.6 ± 1.4	25.6 ± 5.1	57.6 ± 14.6	16.4 ± 10.6	26.0 ± 7.6
T3	16.7 ± 1.4	31.3 ± 3.6	97.0 ± 3.8	0.8 ± 0.8	2.3 ± 3.8
T4	19.6 ± 1.4	29.0 ± 3.6	92.3 ± 4.0	1.0 ± 0.6	6.9 ± 4.6

The initiation of feeding was evident by day 3 in all treatments. At this stage visual observation indicated that about 5-10% of the fry had started feeding in T1, about 30-40% in T2, and the majority in T3 and T4. Fish congregated as a loose school toward the top of the tanks awaiting food, but once it was dispensed they fed throughout the water column.

Figure 6.1. The experimental set-up, showing the conical rearing containers, automatic feed dispensers and electronic control boxes for each dispenser.

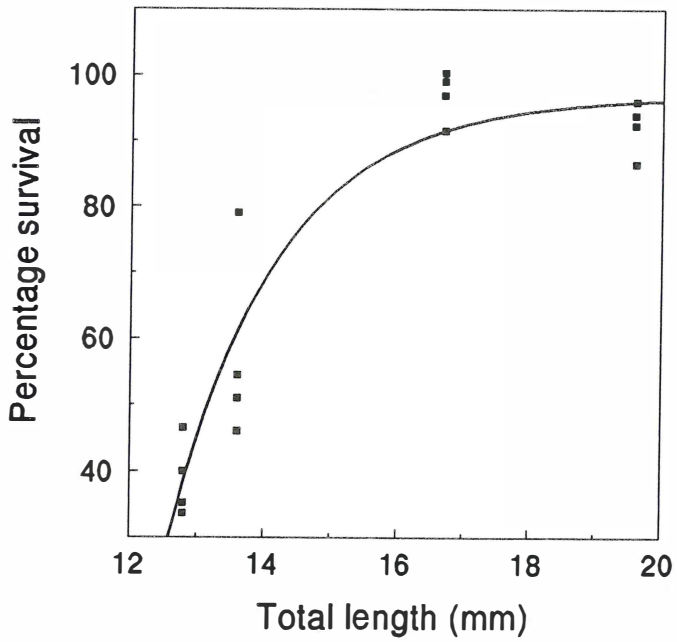

Figure 6.2. Percentage survival through weaning onto dry diets for four different size groups of barramundi fry.

Weaker, non-feeding fish were darkly pigmented. In T1, there was a marked increase in mortality of these fish during days 5-7, peaking at day 6. There was little mortality thereafter, as virtually all remaining fish were feeding. There was no similar peak in mortality of non-feeding fish in the other treatments. Cannibalism was more severe in T2 than in the other treatments.

The aeration set-up was effective in lifting the sinking food particles back into suspension, thus giving the fry longer exposure to the food at each feeding period than would have been the case if the food was allowed to fall through the water column to the base of the tanks. A slimy, organic film developed each day on the walls of the tanks, as a consequence of the high organic load. The circular current swept food particles onto the film, where they attached, effectively becoming unavailable to the fry. This effect became more pronounced as the film redeveloped after cleaning.

DISCUSSION

The experimental design used in this study was compromised by the fact that the treatments were tested on batches of fish from different spawnings. It is a common phenomenon in fish culture that larval fish originating from different parents, or even from the same parents but different spawning events, may exhibit different levels of fitness (often referred to as 'larval quality'). Quality is usually appraised in terms of vigour (swimming strength, ability to orientate, response to stimuli) and health (absence of mortalities, disease and deformities). To avoid 'experimental noise' due to variation in fitness, researchers generally aim to test treatments on high quality fish from the same spawning. We were unable to use fish from the same spawning because of a shortage of experimental tanks and automatic feeders. Nevertheless, we are confident that the survivals determined in the different treatments faithfully reflect the influence of initial size, because of the consistency within treatments and similar survivals shown in other weaning events which were not part of this experiment.

The survival curve for barramundi fry presented in Figure 6.2 indicates that greater than 90% survival during weaning onto dry diets can be expected when using fish larger than 16.2 mm TL at the initiation of weaning. The shape of the curve confirmed our hypothesis that survival through the weaning period is enhanced if weaning is delayed until the fish have adopted the lurking predator mode of feeding. This is also supported by the increasing rapidity with initial size with which feeding on the dry diet was commenced by the majority of fish. The asymptotic shape of the curve indicates that there is negligible gain in survival if weaning is initiated with fish larger than about 16-17 mm TL. The results in this experiment are similar to those of Verreth and van Tongeren (1989) who showed that larvae of the African catfish, *Clarius gariepinus*, could

be successfully adapted onto dry diets after two days feeding on live foods, and that delaying weaning beyond that stage did not improve survival.

Cannibalism was more severe in T2 than in the other treatments. This was probably due to the comparatively wide size range of fish in this treatment at the start of the trial. Moreover, the rapid adaptation of T3 and T4 fish onto the dry diet would have mitigated against the establishment of cannibalism.

Rearing of barramundi fry on live foods is a costly process, as it requires commitment of both manpower and facilities. Hence, hatchery operators generally attempt to wean the fry onto artificial diets as soon as practicable, which is at about 10 mm TL (Awang 1987; MacKinnon 1987; Maneewong 1987; Tucker *et al.* 1988). This study has shown, however, that survival through the weaning period is considerably enhanced if the initiation of weaning is delayed until the fry are 16-17 mm TL. Hatchery operators need to interpret these results from a commercial perspective, but in our experience the economic advantage of survival of say 90% with 16-17 mm TL fry would outweigh the reduced rearing costs associated with weaning fish at 13 mm TL and concomitant survival of say 50%. We suggest the disparity would be even greater if weaning is initiated when the fry are 10 mm TL.

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Chapter 7

EFFECTS OF PHOTOPERIOD ON GROWTH AND FEEDING PERIODICITY OF BARRAMUNDI *Lates calcarifer* FRY

Authors: C.G. Barlow, L.J. Rodgers and P. Clayton

ABSTRACT

An experiment was conducted to determine the effect of extended periods of light on the growth, survival, feeding pattern and daily food consumption of barramundi fry reared in a freshwater hatchery. There was no significant difference in growth or survival of fry, initially 11-12 mm total length, in either 12, 18 or 24 hours light. Fish exposed to 12L/12D photoperiod fed continuously during daylight, and ceased feeding in darkness. Under continuous daylight conditions, fish fed throughout the normal daytime period, but ceased feeding at a time corresponding to the normal onset of darkness; feeding started again near midnight. Daily food consumption for 34 mm fish was approximately 40% more in continuous light than in 12L/12D photoperiod. The results clearly indicate that there is no advantage to be gained by rearing barramundi fry in extended light regimes.

INTRODUCTION

Barramundi or sea bass, *Lates calcarifer* (Bloch) (Centropomidae), is a highly valued catadromous fish which is widely distributed within the tropical Indo-Pacific region (Greenwood 1976). In many countries within the region it is reared for farming purposes (Copland and Grey 1987). In Australia it is bred both for grow-out on farms and for stocking recreational fisheries (Pearson 1987, Rutledge *et al.* 1990).

There is surprisingly little literature on the biology of barramundi larvae and fry as it relates to survival and growth under hatchery conditions (although there is considerable published information on rearing procedures). Pearce (1991) showed that artificially extending daylight during larval rearing resulted in increased growth due to greater food consumption, but that it had no effect on survival. Barlow *et al.* (1993) reported that barramundi fry were visual feeders, taking food throughout the day, with a peak in feeding activity at dusk. The fry continued feeding at a reduced level under moonlit conditions, but ceased feeding in total darkness.

Based on this information, we hypothesised that artificially increasing the day length during hatchery rearing may increase the growth rate of barramundi fry. Consequently, we undertook a study to determine if photoperiod had any effect on the growth, survival, feeding pattern and daily food consumption of barramundi fry reared in a freshwater hatchery.

MATERIALS AND METHODS

The fish

The fish were bred from eggs stripped from wild fish captured on the spawning grounds in the Hay River, Weipa (12°34'S, 142°53'E). The larvae were reared at the Northern Fisheries Research Centre, Cairns. After metamorphosis, at about 18 days old, the fish were acclimated to fresh water over a 24 hour period and transferred to the Walkamin Research Station. They were maintained in fresh water for at least 5 days prior to starting the growth and survival experiment, and at least 25 days prior to starting the duration of feeding experiment. Two trials were conducted within each experiment, using fish from separate spawnings.

Effect of photoperiod on growth

The first trail was designed to test the effect on growth of 12 hours light and 12 hours dark (12L/12D) and continuous lighting (24L/0D) in combination with

food being available for either 12 or 24 hours. Treatments were as follows:

Treatment 1 : 12L/12D, food available for the 12 daylight hours
Treatment 2 : 12L/12D, food available 24 hours
Treatment 3 : 24L/0D, food available 12 hours
Treatment 4 : 24L/0D, food available 24 hours

The second trial was designed to test the effect on growth of 12L/12D (treatment 1), 18L/6D (treatment 2) and 24L/0D (treatment 3) light regimes, with food available continuously.

All treatments were replicated 5 times, with 20 fish per replicate. Each replicate consisted of an aquarium tank 900 x 350 x 300 mm^3, supplied with an independent biological filter and aeration. Artificial shelter, in the form of 12 strips (30 x 400 mm^2) of black plastic mesh (1 mm^2) was suspended in each aquarium. Temperature was maintained at 29.0 $\pm$ 1.0°C.

Lighting was controlled by covering each aquarium with black material and positioning one 15 watt incandescent globe 25 cm above the surface of the water at the centre of the aquarium. Automatic time switches turned lights on and off at either 0700 hours and 1900 hours (trial 1, treatments 1 and 2; trial 2, treatment 1) or 0700 hours and 0100 hours (trial 2, treatment 2) respectively. Light intensity at the water surface varied from 300 lux at the ends of the aquaria to approximately 1100 lux directly under the globes.

Equal aliquots of live zooplankton were added to each aquaria at 0700 hours and 1600 hours. The zooplankton was harvested from a pond using airlifts and a 250 um net. The amount fed was sufficient for excess food to be available continuously during the feeding period. To maintain the zooplankton within the aquaria, inlet water to the biological filters was strained through 200 um filter boxes. In the 12 hour food availability treatments in trial 1, the inlets to the biological filters were removed from the filter boxes at 1900 hours, which effectively removed all zooplankton from the aquaria within one hour.

Fish were allocated randomly at the start of each trial, with a subsample retained for weighing and measuring. Total lengths (TL) and wet weights (Wt) at the start of each trial were as follows :-

Trial 1	TL (mean $\pm$ s.d.)	11.9 $\pm$ 1.4 mm, range 10.0-16.1 mm, n = 46
	Wt (mean $\pm$ s.d.)	23.0 $\pm$ 8.7 mg, range 10.0-50.0 mg, n = 46
Trial 2	TL (mean $\pm$ s.d.)	11.0 $\pm$ 1.1 mm, range 9.1-13.0 mm, n = 50
	Wt (mean $\pm$ s.d.)	21.3 $\pm$ 7.1 mg, range 9.0-36.0 mg, n = 50

Each trial was run for 13 days, after which all fish were weighed and measured. Two-way analysis of variance (ANOVA) was used to analyse growth data in trial 1, and one-way ANOVA was used for growth comparisons in trial 2.

In Trial 1, 10 fish from each treatment were killed by immersion in 0°C water and fixed in 70% alcohol. Longitudinal section mounts stained with haematoxylin and eosin were made and examined to determine if histological abnormalities were induced by continuous lighting.

Effect of photoperiod on feeding periodicity

Two trials were conducted with different sized fish to determine the patterns of food consumption over a 24 hour period for fish exposed to 12L/12D and 24L/0D light regimes. Approximately 80 fish for each treatment were placed into aquaria set up as described above, and acclimated to the experimental conditions for 3 days prior to the trials. The TLs of the fish used in each trial were as follows.

Trial 1	TL (mean $\pm$ s.d.)	33.5 $\pm$ 3.3 mm, range 24.7-39.9 mm, n = 64
Trial 2	TL (mean $\pm$ s.d.)	51.7 $\pm$ 5.0 mm, range 41.3-62.3 mm, n = 64

Excess live zooplankton was fed one hour prior to sampling, which was every three hours for 24 hours. Eight fish per treatment were sampled on each occasion. Immediately after sampling the fish were killed by immersion in 0°C water, and preserved in 70% alcohol. Within one week, the stomach contents were removed, and the dry weights of fish and stomach contents were recorded after drying at 60°C for 24 hours. The stomach fullness indexes for each sampling period were calculated from the formula

$$\text{Stomach fullness index} = \frac{\text{dry weight of stomach contents}}{\text{dry weight of fish}} \times 10^2$$

For the fish in trial 1, the percentage body weight eaten per day was determined from the formula

$$\text{Daily ration} = \frac{\text{24 hours average stomach fullness index}}{\text{number of hours to evacuate food from stomach}} \times 24$$

The number of hours for stomach evacuation for barramundi with a mean total length of 34 mm was taken as 1.5 hours (Barlow *et al.* 1993). (The percentage body weight eaten per day not calculated for fish in trial 2, because the stomach evacuation rate for 52 mm barramundi is not known).

RESULTS

Effect of photoperiod on growth

Final lengths, weights and survival of barramundi fry in trials 1 and 2 are listed in Tables 7.1 and 7.2 respectively. In trial 1, analysis involved the partitioning of treatment effects into photoperiod, food availability and interaction effects. The only effect which was statistically significant was that of food availability. Having food available for 24 hours increased length by 0.7 mm ($P = 0.032$) and increased weight by 19 mg ($P = 0.52$). Note that the latter falls just short of the conventional 5% significance level. The lack any interaction indicated that this increase was unrelated to photoperiod. This was confirmed in trial 2, in which there was no significant difference in the length or weight of fish in the three photoperiod treatments.

Mean survival in trial 1 was in the range 93-97%, and in trial 2 it was 66-76%. There was no significant difference between the survivals in the various treatments within either trial.

Histological examination of specimens from trial 1 revealed no abnormalities or lesions on any fish.

Effect of photoperiod on feeding periodicity

The patterns of food consumption over 24 hours were different between the two treatments, with the same patterns being exhibited by both small (trial 1) and large (trial 2) fish. Fish exposed to a 12L/12D regime fed continuously during the lighted period, but ceased feeding during darkness (Fig. 7.1A). Feeding appeared to taper off after 1500 hours, and food remaining in the stomach at 2100 hours was well digested, indicating that feeding ceased with the onset of darkness.

Fish exposed to continuous light also showed the same trend of decreased feeding after 1500 hours, and at 2100 hours all had empty stomachs. Thereafter, the fish started feeding again (Fig 7.1B), in marked contrast to those in the 12L/12D light regime.

The 24 hour average stomach fullness index for the small fish (trial 1) was 1.65 for the 12L/12D treatment and 2.28 for the 24L/0D treatment. Using these data, the daily ration in the 12L/12D treatment was 26.4 % and in the 24L/0D it was 36.5%. That is, the fish exposed to continuous lighting consumed approximately 1.4 times more food than the fish in the 12L/12D regime.

Table 7.1.

Mean lengths (TL, mm), weights (Wt, mg) and percentage survivals (and standard errors) of barramundi after being exposed to various photoperiod and food availability treatments for 13 days. Initial size was TL = 11.9 ± 1.4 mm, Wt = 23.0 ± 8.7 mg.

Treatment	Mean TL ± S.E. (mm)	Mean Wt ± S.E. (mg)	% Survival Mean ± S.E.
T1 = 12L/12D, food 12 hours	30.5 ± 3.2	396.5 ± 109.9	93 ± 5.7
T2 = 12L/12D, food 24 hours	31.0 ± 3.1	412.2 ± 113.6	95 ± 0.0
T3 = 24L/0D, food 12 hours	30.7 ± 3.0	400.6 ± 106.4	95 ± 6.1
T4 = 24L/0D, food 24 hours	31.6 ± 2.9	423.7 ± 107.8	97 ± 2.7

Table 7.2.

Mean lengths (TL, mm), weights (Wt, mg) and percentage survivals (and standard errors) of barramundi fry after being exposed for 13 days photoperiod regimes of 12L/12D, 18L/0D and 24L/0D, with food continuously available. Initial size was TL = 11.0 ± 1.1 m, WT = 21.3 ± 7.1 mg.

Treatment	Mean TL ± S.E. (mm)	Mean Wt ± S.E. (mg)	% Survival Mean ± S.E.
T1 = 12L/12D	30.3 ± 4.5	385.8 ± 175.3	66 ± 11.4
T2 = 18L/6D	29.4 ± 4.1	337.7 ± 142.6	73 ± 10.4
T3 = 24L/0D	30.4 ± 4.2	374.6 ± 166.7	76 ± 2.2

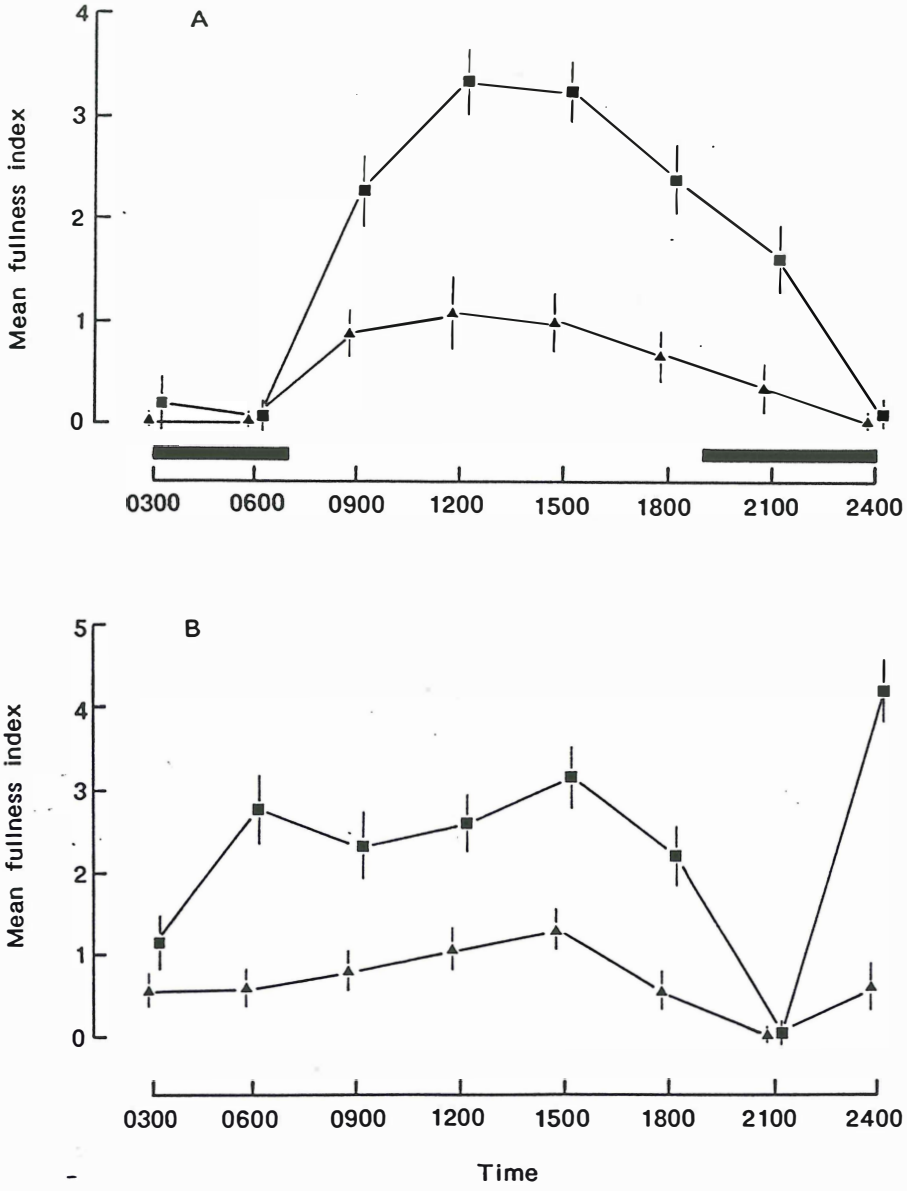

Figure 7.1. Stomach fullness indexes for two size groups of barramundi fry exposed to 12L/12D (A) and 24L/0D (B) light regimes, with food continuously available. Each datum is the mean ± s.e. (n = 8). The solid bar indicates the period of darkness. □ = fry TL 33 ± 3.3 mm (mean ± s.d.); * = fry TL 51.7 ± 5.0 mm (mean ± s.d.).

DISCUSSION

The results of this study clearly show that there is no advantage to be gained by rearing barramundi fry under extended light regimes. While fry in extended daylight did not show any adverse effects (in either survival or morphological development), they grew no faster than those in normal daylength light regimes. This was despite the fact that fry in 24 hours light consumed approximately 40% more food than did those in 12 hours light (with food continuously available in both cases). Presumably, the extra intake under continuous light was expended as non-productive energy associated with increased activity of the fish.

Barramundi fry reared in outdoor ponds have a distinct feeding pattern, with food being consumed throughout the day with a distinct peak in food intake at dusk. The fry do not feed in total darkness (Barlow *et al.* 1993). In the present study, in which we did not emulate dawn/dusk lighting, peak feeding in the 12L/12D regime was in the early afternoon, with intake decreasing in the late afternoon and ceasing in darkness. A similar afternoon pattern was maintained by fish in continuous light, with all fish having empty stomachs at 2100 hours. Prior to 2400 hours, feeding resumed in continuous light (Fig. 7.1B), in contrast to fish in the 12L/12D cycle. The maintenance of a modified feeding pattern in continuous light and in the absence of other external cues indicates that the feeding pattern is only partly controlled by light, and that other, presumably innate or genetic, controls are involved.

The results presented here and those of Pearce (1991) (Table 7.3) indicate that continuous light is advantageous for growth of larval barramundi during their first 8 days, but thereafter it becomes less important and has no effect after metamorphosis (which is at 11 mm TL, or 20-25 days of age). Information on the effect of extended light periods on other species (Table 7.3) indicates a similar trend. Extended periods of light during larval rearing are generally beneficial for growth (but not necessarily survival). After metamorphosis, however, it seems that growth of juvenile fishes is usually the same in both normal and extended light regimes.

Table 7.3.

Literature reports on the effect of extended light periods on the growth and survival of larvae and juveniles of several species of fin fishes. (Salmonid fishes are not included, because with these anadromous species the effect of photoperiod interacts with the state of physiological development and time of year).

Species	Developmental Stage	Treatment	Response	Reference
Siganus guttatus	First-feeding larvae, 0-7 days	Continuous light vs natural light/dark cycle	Growth rate and survival better in continuous light.	Duray and Kohno 1988
Lates calcarifer	Larvae, 2-20 days	12, 18 and 24 hour light periods	Days 2-10 (rotifer feeding) - growth significantly better in 24 hours light, survival not significantly different. Days 8-20 (*Artemia* feeding) - growth significantly better in 16 and 24 hour light, survival not significantly different.	Pearce 1991
Dicentrarchus labrax	Larvae, 0-30 days	12, 18 and 24 hour light periods	Maximum growth at 18 hours light, maximum survival at 12 hours light.	Barahona-Fernandes 1979
Nautichthys oculofasciatus	Larvae, 0-38 days	13 and 24 hour light periods; simulated natural photoperiod (dawn/dusk plus low intensity light at night).	Survival in 24 hours light and simulated natural photoperiod significantly better than in 13L/11D.	Marliave 1977
Sparus aurata	Larvae, 0-70 days	12 and 24 hour light periods	Survival and growth best in continuous light.	Tandler and Helps 1985
Solea Solea	Larvae and juveniles to 3 months old	12, 18 and 24 hour light periods	Larvae - survival not significantly different, growth better in 18 and 24 hours light. Juveniles - survival and growth not significantly different.	Fuchs 1978
Mylio macrocephalus	Larvae and juveniles 0-108 days	13, 18 and 24 hour light periods	Larvae - growth best under continuous light. Juveniles - growth not significantly different.	Kiyono and Hirano 1981
Sebastes dipoproa	Juveniles, 30-55 mm standard length.	12 and 16 hour light periods.	Growth rates better in 16 hour light.	Boehlert 1981

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Chapter 8

EFFECTS OF DENSITY AND INITIAL GROWTH RATES ON THE PRODUCTION OF JUVENILE BARRAMUNDI *Lates calcarifer* IN CAGES

Authors: L.J. Rodgers and J.P. Bloomfield

ABSTRACT

This study was undertaken to provide information on the effects of density and initial growth rates of fry on the production of juvenile barramundi *Lates calcarifer* in cages. Juvenile barramundi of the Cairns strain were graded into three categories of poor, average and good quality, based upon their initial growth performance during the pre-experimental period. Average and good quality fish of 7.6 and 11.1 g mean weight were subsequently cultured at densities of 120, 180, 240 and 360 fish/m^3 for a period of 103 days. Mean temperatures for each trial period varied between 22 and 24°C, while salinities increased from 5.5 to 28.2% . Average quality fish achieved final mean weights of 71.2, 72.9, 67.2, and 59.3 g while good quality fish achieved corresponding mean weights of 80.7, 82.8, 82.3, and 83.6 g at densities of 120, 180, 240 and 360 fish/m^3 respectively. The weight gains of average quality fish cultured at 360 fish/m^3 were significantly lower ($P<0.05$) than similar fish cultured at 120, 180 fish/m^3 or 240 fish/m^3. Average quality fish cultured at 240 fish/m^3 also displayed a significantly lower weight gain ($P<0.05$) than when cultured at 120 fish/m^3. There was no significant difference in the weight gains of good quality fish at the different densities 120, 180 and 240 fish/m^3. The pooled data for both groups indicated that total production of fish at 360/m^3 was significantly greater ($P<0.05$) than fish cultured at 120/m^3 or 180/m^3 but not 240/m^3. The results show that growth rates and seasonal production of smaller juvenile barramundi (< 60 g) of average and good quality may be maximised at culture densities of 180 and 360 fish/m^3 respectively. High cage densities of 360 fish/m^3 may be suitable for the growth of larger fish (>60 g) to market size during autumn, providing adequate water quality can be maintained. An alternative management procedure, which did not consider larval quality, suggested that production was maximised at culture densities of 240 to 360 fish/m^3.

INTRODUCTION

The culture of barramundi, *Lates calcarifer* (Centropomidae), is a new industry of northern Australia, developing in a region located near the southern limit of the species natural distribution (Grey 1987). Seasonal factors have resulted in the selection of high density cage culture techniques to capitalise on the rapid growth of *L. calcarifer* during the warmer months, while reducing the costs of overwintering fish when little growth occurs.

Although this culture strategy increases the production of *L. calcarifer* from each cage unit in tropical waters (Sakaras 1987), it may also compromise the growth and survival of individual fish. High fish densities in confined environments are often associated with reduced growth rates and increased mortality (Maneewong *et al.* 1986), elevated levels of metabolites and pathogens (Soderberg and Krise 1986) and increased competition for food (Holm *et al.* 1990) and oxygen (Wu and Lee 1989).

In Australian waters, these stress factors are also seasonally exacerbated by low water temperatures which may reduce growth rates (McKinnon 1987), appetite and disease resistance (Chapters 9 and 10, this report). The combined interaction of these factors limits the production of this species in north-eastern Australia during the cooler winter months. Therefore, it is particularly important from an economic perspective that the growth of *L. calcarifer* in more southern tropical waters is not unnecessarily restricted by density effects, when suitable temperatures for growth prevail.

Significantly, many of these stress factors do not act equally upon all fish which originate from a single spawning. Investigations upon carp (*Cyprinus carpio*) have identified poor, average and good performing groups of fish that display variable growth rates in accordance with the interaction of a genetic factor with culture densities or other environmental factors (Wohlfarth and Moav 1972, Moav and Wohlfarth 1973). Similarly, Degani and Levanon (1983) classified wild caught elvers (*Anguilla anguilla*) into three categories of quality according to their initial growth performance at low to moderate culture densities. Only the best quality elvers were considered suitable for further culture.

In contrast, only the poorest quality juvenile *L. calcarifer* are initially culled by Australian producers, often to remove fish with spinal or swimbladder abnormalities. The remaining fish are then ongrown at similar high stocking densities. It is possible that this practice of culturing all of the remaining fish at similar high densities may compromise individual growth to varying degrees in accordance with larval quality, resulting in decreased production.

This study was therefore undertaken to determine appropriate culture densities for

the production of juvenile *L. calcarifer* of different quality, but derived from the same spawning.

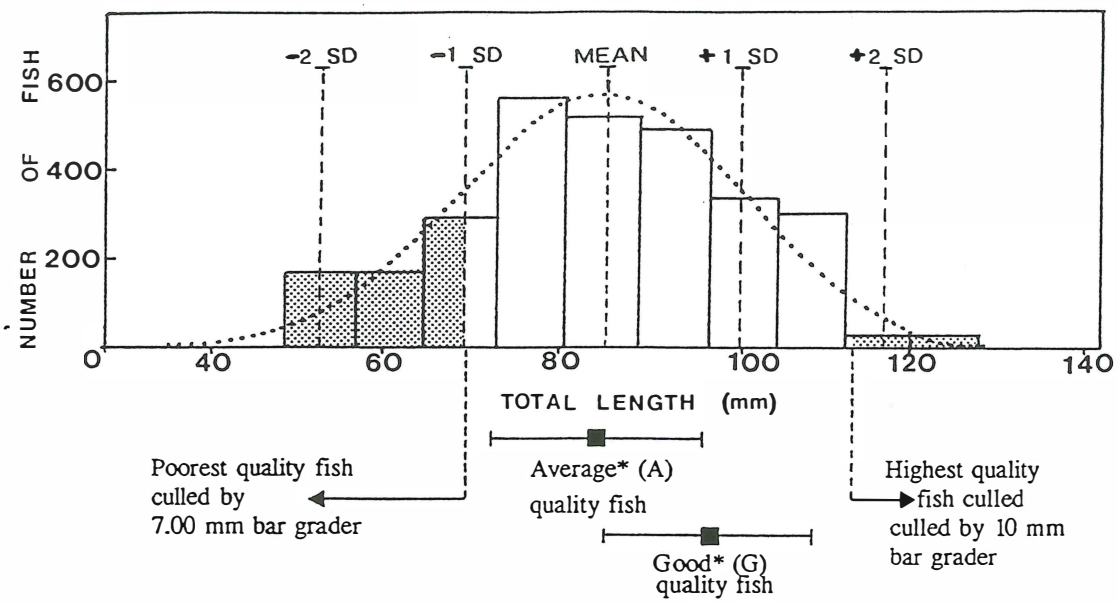

Figure 8.1. Selection of experimental fish groups of average (A) and good (G) quality. * mean $\pm$ 2 s.d.

METHODS AND MATERIALS

Stock history and selection

Eight thousand barramundi larvae were obtained from an induced mid-January spawning of captive "Cairns" broodstock and intensively reared in a saltwater hatchery (Rimmer *et al.* 1991, Pearce 1991). Juveniles of 10-12 mm total length (TL) were subsequently acclimated to freshwater and ongrown in tanks using freshwater zooplankton, prior to being weaned onto a dry crumble diet at 20-30 mm TL, and further ongrown in cages. Juvenile fish were graded as required with bar graders to reduce cannibalism. All fish were maintained at similar densities of three to five fish/litre and overall survival to a weaned juvenile stage was 56.2%.

The different quality of juveniles was indicated by the variation in individual size attained prior to the experiment. Consequently, the poorest quality juveniles were culled after ongrowing by passing through a 7.00 mm bar grader while larger, 'better quality' fish were retained for experimental purposes (Figure 8.1). These larger juveniles were subsequently graded into two experimental fish groups of

average (A) and good quality (G) by either passing through or being retained by an 8.00 mm bar grader respectively (Figure 8.1). A small number of the largest, highest quality fish were also culled by a 10 mm grader in order to decrease the risk of cannibalism during the trial.

Experimental design

The trial began during the mid-autumn month of May. Juvenile *L. calcarifer* from each of the two experimental fish groups (A) and (G) were stocked into four floating 1 m^3 cages at densities of 120, 180, 240 and 360 fish/m^3. The mean size (mean $\pm$ s.d.) of average and good quality fish groups measured 83.3 $\pm$ 6.0 mm TL (7.6 $\pm$ 1.4 g) and 97.2 $\pm$ 6.0 mm TL (11.1 $\pm$ 1.9 g) respectively. A total of eight cages were used and initial cage biomass varied between 0.87 and 3.99 kg/m^3.

Each cage of 1.35 m^2 surface area and 10 mm mesh size was aerated with a single airstone and assigned a random position about a pontoon located within a brackishwater pond near Innisfail, Queensland. Pond water was partially exchanged on a daily basis throughout the experimental period of 103 days in order to maintain water quality.

Temperature was recorded daily with a max/min thermometer positioned at a depth of 90 cm. Dissolved oxygen concentration, salinity and pH were also recorded daily throughout the trial.

Feeding procedure

A commercially available dry barramundi diet[1] was fed to fish at 4.5% of body weight per day. The feed was offered twice daily, at approximately 10.00 am and 6.00 pm with the feed quantity revised at the end of each trial period of approximately 20 days.

Data collection and analysis

A sample of 50 fish was taken from each cage at the end of each trial period. The fish were weighed to the nearest 0.1 g and measured to the nearest mm (TL) before being placed back into their original cage. Mortalities were recorded daily and all cages were routinely spray cleaned at the conclusion of each trial period.

[1] Barramundi Starter Diet manufactured by Aquafeed Products, Australia to the specifications of formulae no. 521007 of proximate composition as follows : crude protein 50%; lipid 10%; carbohydrate 15.3%; ash 12.7%; fibre 3%; and moisture 8%. The use of this product for research purposes does not imply endorsement of the company or its products.

Daily specific growth rates (G_w % day^{-1}) were calculated for each trial period using the formula

$$G_W = \left(\frac{Log_e\ W_2 - Log_e\ W_1}{t\ (days)}\right)^* 100 \qquad \text{(Elliot 1975)}$$

where W_1 and W_2 are the mean individual weights at the beginning and end of each period respectively.

Mean individual weights ($\overline{W}$) were calculated for each period using the formula

$$\overline{W} = \frac{W_2 + W_1}{2} \qquad \text{(Elliot 1975)}$$

The mean biomass ($\overline{B}$) for each growth period was also calculated using a similar formula.

ANCOVA procedures for the comparison of slopes and intercepts were based upon the methods outlined by Zar (1984) and included Dunnet's test for multiple comparisons. Duncan's Multiple Range Test was used for the comparison of means in association with ANOVA techniques.

Table 8.1. Environmental conditions of the experimental, brackishwater fish pond during June to September, 1990.

Growth period no.	1	2	3	4	5
Mean temp °C	23.9	22.1	23.8	23.6	24.0
Mean min temp (± SD)	23.11 ± 1.07	21.34 ± 0.58	23.07 ± 1.48	22.56 ± 1.08	23.02 ± 1.08
Mean max temp (± SD)	24.65 ± 0.92	22.91 ± 1.07	24.47 ± 1.46	24.70 ± 1.05	25.04 ± 1.08
Dissolved oxygen ppm*	8.46 (7.2-10.4)	8.08 (6.9-9.6)	7.52 (6.8-8.6)	7.89 (6.7-8.6)	6.13 (4.9-7.3)
Salinity ppt*	9.9 (5.5-12.3)	13.7 (12.1-15.1)	17.17 (15.6-18.9)	21.6 (19.0-25.4)	26.33 (23.7-28.2)
pH*	7.85 (6.8-8.5)	8.33 (7.4-8.9)	7.8 (7.4-7.9)	8.33 (7.9-8.7)	7.7 (7.5-8.6)

* Mean and range

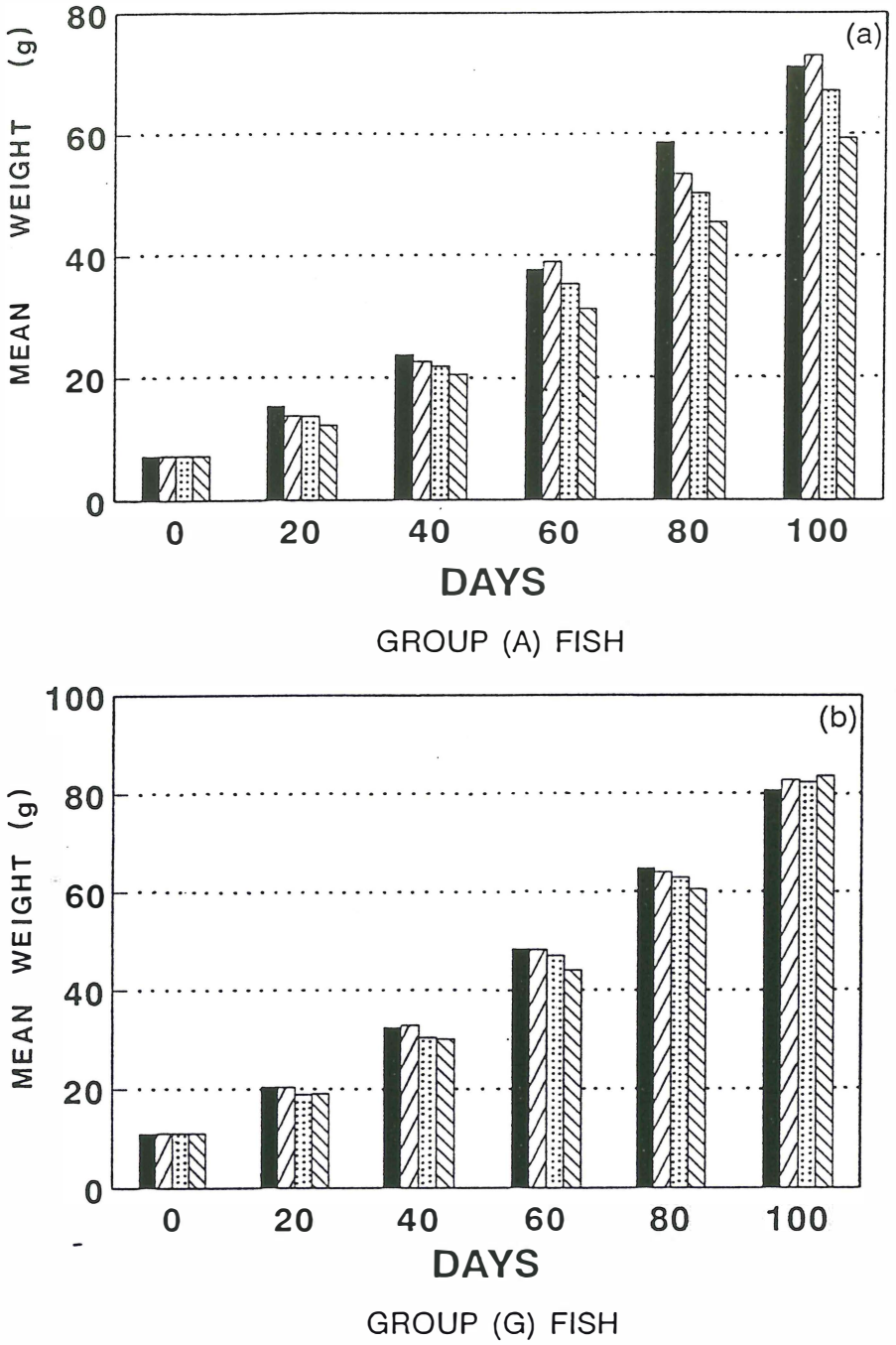

Figure 8.2. Cumulative Weighty Gain of Average (A) and good (G) quality fish groups at culture densities of 120, 180, 240 and 360 fish/m^3.

RESULTS

Environmental conditions

Prolonged overcast weather and the ability of ponds to absorb solar radiation resulted in an unusually warm autumn and winter which was characterised by relatively stable mean temperatures throughout the experiment. Mean temperatures of between 23.6 and 24.0°C occurred during growth periods 1, 3, 4 and 5 while a lower corresponding temperature of 22°C occurred during growth period 2 (Table 8.1).

Salinity steadily increased throughout the trial in contrast to dissolved oxygen levels which slowly decreased but always remained above 4.9 ppm. Pond pH values varied between 6.8 and 8.9 (Table 8.1). Nitrite and total ammonia nitrogen levels remained below 0.25 and 0.2 ppm respectively throughout the trial.

Growth

The mean weights observed at the conclusion of each trial period, for average and good quality fish cultured at the various densities, are displayed in Figs. 8.2a and b. The lower densities of 120 and 180 fish/m^3 of both fish groups appeared to display comparatively higher weight gains during the first four growth periods before slowing, relative to other densities, during the final period of the study.

The results of a two way ANOVA (times x density) of the $\log_e$ transformed cumulative weight gains for group (A) and (G) fish during the five trial periods are presented in Tables 8.2 and 8.3 respectively.

Table 8.2. Two-way analysis of variance table for the $\log_e$ transformed cumulative weight gains of group (A) fish for the five trial periods. Values with the same letter were not significantly different ($P > 0.05$) from each other.

Source of variation	SS	df	MS	F	Density fish/m^3	$\log_e$ Mean	Mean (g)
Times	12.7691	4	3.192277	978.59	120	3.2868^a	26.76 g
Density	0.235335	3	0.078445	24.05***	180	3.2179^{ab}	24.97 g
Error	0.003914	12	0.003262		240	3.1493^b	23.32 g
Total	13.043	19			360	2.9940^c	19.97 g

Weight gains were significantly lower ($P < 0.05$) for average quality fish cultured at the highest density of 360 fish/m^3, when compared with the growth of similar fish cultured at the lower densities of 120 and 180 fish/m^3 (Table 8.2). Similarly, the weight gain of the fish at 240 fish/m^3 was significantly lower ($P < 0.05$) than when cultured at 120 fish/m^3 but significantly higher ($P < 0.01$) than when cultured at 360 fish/m^3.

In contrast, good quality fish cultured at the highest density of 360 fish/m³ displayed significantly higher weight gains ($P < .05$) than similar fish cultured at the lower densities of 120 and 180 fish/m³ (Table 8.3). There were no significant differences in the weight gains of fish cultured at 120, 180 or 240 fish/m³.

Table 8.3. Two-way analysis of variance table for the $\log_e$ transformed cumulative weight gain of group (G) fish for the five trial periods. Values with the same letter were not significantly different ($P > 0.05$) from each other.

Source of variation	SS	df	MS	F	Density fish/m³	$\log_e$ Mean	Mean (g)
Times	11.3327	4	2.833277	1311.87	120	3.3413^{a}	28.26 g
Density	0.028784	3	0.009595	4.43*	180	3.3713^{ab}	29.12 g
Error	0.025916	12	0.002167		240	3.4268^{abc}	30.78 g
Total	11.3874	19			360	3.4312^{c}	30.91 g

However, the observed trends in both fish groups during the trial suggested that the growth rates at different densities were being continuously modified by the interaction of density and biomass related factors with the current mean individual weight.

Fortunately, the stable temperature and feeding regimes permitted further investigation of growth rates using regression analysis. Four regression equations were obtained to describe the pooled growth rates of fish at each density as a function of the mean individual weight ($\bar{W}$) during growth periods 1, 3, 4 and 5, when similar mean temperatures of approximately 23.6 to 24°C prevailed (Table 8.4). The results of ANCOVA analysis of the slopes and intercepts of these regressions is presented in Table 8.4.

Table 8.4. Linear regressions describing specific growth rate (individual daily % gain) for pooled data as a function of mean wt (g) during growth periods 1, 3, 4 and 5. Values with same letter were not significantly different ($P > 0.05$) from each other.

Density fish/m³	a ± S.E.	b ± S.E.	r^2	P
120	3.774^{x} ± 0.2192	-0.0405^{x} ± 0.00462	0.93	< 0.001
180	3.372^{xy} ± 0.1768	-0.0323^{xy} ± 0.00377	0.92	< 0.001
240	3.181^{xy} ± 0.1354	-0.0295^{xy} ± 0.00301	0.94	< 0.001
360	2.702^{y} ± 0.1697	-0.0211^{y} ± 0.00398	0.82	< 0.005

Individual regressions for each density suggested that the greatest difference in growth rates between densities coincided with fish of small individual weight. These differences in growth rates between densities appeared to diminish as the fish approached individual weights of 50 to 60 gms (Figs. 8.2a, b).

Multilinear regression techniques upon this same data revealed the following significant descriptive relationship ($P < 0.01$) for specific growth rates as a function of density and mean individual weight $\bar{W}$ during growth periods 1, 3, 4 and 5.

$$G_w = 3.69 - .0336\ \bar{W} - 0.00132\ \text{Density}.$$

The effect of increased stocking rates upon the growth rate of juvenile *L. calcarifer* was also examined from a biomass/m³ perspective (Fig. 8.3). Although a single, significant linear regression could be obtained by examining individual growth rate as a function of the log transformed mean cage biomass for growth periods 1, 3, 4 and 5, it was apparent that the data were heteroscedastic. Further examination suggested that the additional variable of biomass/m³ interacted with mean individual fish weight at each density to produce the four significant linear regressions ($P. < 0.05$) which are displayed in Fig. 8.3 and listed in Table 8.5.

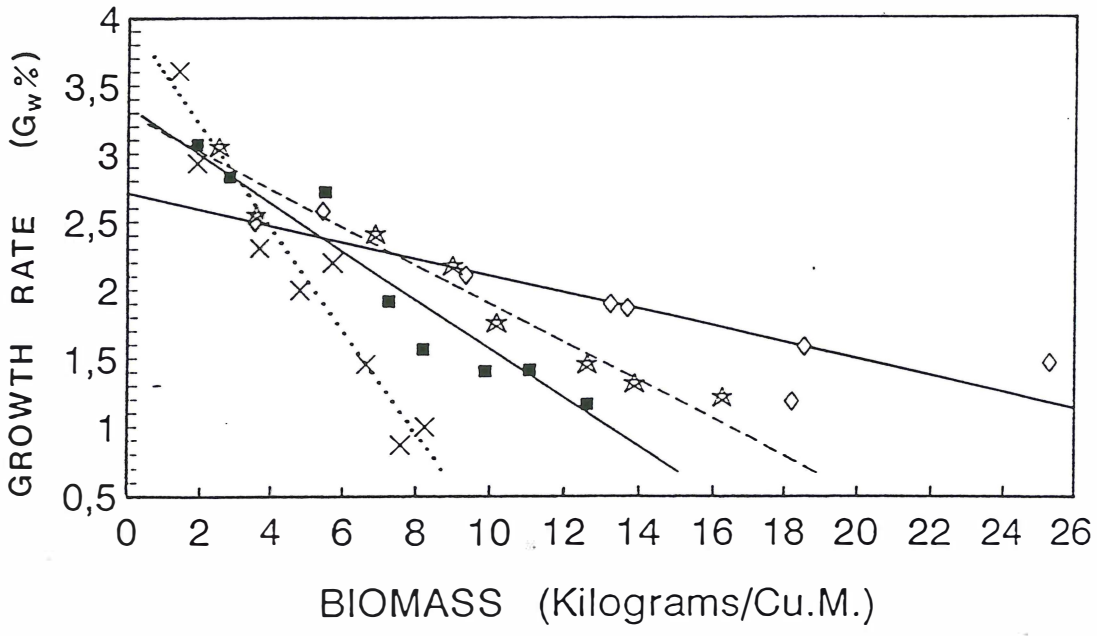

Figure 8.3. Linear regressions of specific growth rate as a function of mean cage biomass for pooled data derived from average and good quality fish groups during trial periods 1, 3, 4 and 5.
120 fish/m³ 180 fish/m³ 240 fish/m³ 360 fish/m³

Table 8.5. Linear regressions of specific growth rate (individual daily % weight gain) as a function of mean cage biomass (kg) for pooled data at each density during trial periods 1, 3, 4 and 5. Values with same letter were not significantly different from each other.

Density fish/m³	a ± S.E.	b ± S.E.	r^2	P
120	3.826 ± 0.2185	-0.0003574^x ± 0.0000396	0.93	< 0.001
180	3.408^x ± 0.1731	-0.0001888^y ± 0.000211	0.93	< 0.001
240	3.226^y ± 0.1260	-0.0001320^y ± 0.0000121	0.95	< 0.001
360	2.7113 ± 0.1753	-0.0000605^z ± 0.0000119	0.82	< 0.0025

Mortality

A low incidence of fish mortality occurred in all cages during the last thirty days of the trial but final survivals from all cages varied between 92 and 97%. There were no significant differences in mortality between culture densities or fish groups.

Initially, infected fish displayed a slight abdominal swelling, often located just anterior to the anus. Abdominal distension increased as the disease progressed, to be successively followed by a loss of buoyancy, peritonitis and death.

Dissection revealed visceral adhesions between omental tissues and the peritoneum. White, ellipsoidal abscesses of 5-12 mm diameter were sometimes present on the liver, kidney and intestinal serosa. Larger abscesses were composed of an outer wall of fibroblasts surrounding an amorphous, caseous medulla in which unidentified gram-ve rods were present.

Vibrio harveyi, V. parahaemolyticus, Chromobacterium sp. and other unidentified bacteria were present in the abdominal cavity and viscera of more advanced infections associated with peritonitis (I. Anderson and J. Norton, Oonoonba Veterinary Laboratory, pers. comm. 1991).

The disease syndrome was concluded to be of bacterial origin, possibly associated with unidentified stress factors. Mortalities abated following the inclusion of oxytetracyline in the diet at 70 μg/kg fish/day for a period of ten days.

Production

ANOVA of pooled data from average and good quality fish indicated that the production of fish from cages stocked at 360 fish/m³ was significantly greater ($P < 0.05$) than fish cultured at 120 and 180 fish/m³ but not 240 fish/m³ (Table 8.6).

Table 8.6. One-way ANOVA table of total production of pooled values for fish groups (A) and (G) cultured at each density. Values with the same letter were not significantly different ($P > 0.05$).

Source of variation	SS	df	MS	F	Density fish/m³	Mean (g)
Block	1.24E+07	1	1.24E+07	2.02	120	7433.78[a]
Treatment	2.07E+08	3	6.91E+07	11.22*	180	11770.55[a]
Error	1.85E+07	3	6161504		240	14753.8[ab]
Total	2.38E+08	7			360	214272[b]

DISCUSSION

Juvenile quality influenced individual weight gains at selected densities. Average quality fish cultured at the lower densities of 120 and 180 fish/m³ exhibited significantly higher weight gains than when cultured at 360 fish/m³ (Table 8.2). Similarly, these fish also displayed significantly higher weight gains at 240 fish/m³ than when cultured at 360 fish/m³. Correspondingly, good quality fish did not display similar significant differences in weight gains between culture densities of 120 to 240 fish/m³ (Table 8.3). Therefore, the growth rates of average quality fish appeared to be more susceptible to density associated effects than fish of a higher quality.

Our results also suggested that the greatest reduction in growth rates, due to the effects of density and biomass coincided with the high density culture of young fish of small body weight (Figs. 8.2 and 8.3, Table 8.4). Mean values obtained from linear regressions (Table 8.4) of pooled data suggested that the culture of 10 g fish at 360 fish/m³ could initially result in a possible 25% reduction of the comparative growth rate achieved by the same fish, simultaneously cultured at 120 fish/m³.

The present investigation suggests that the difference in growth retardation at high densities diminished with both increasing fish quality and individual size. Larger fish of >60 g in both fish groups appeared to display comparatively improved weight gains when cultured at higher densities during the final trial period (Fig. 8.2a, b; Table 8.4). The interactive effects of fish size, biomass and density on growth rate, which is suggested by the current study may implicate some change in

fish behaviour or physiology.

Similar findings of growth retardation have been reported for the high density culture of rainbow trout, *Oncorhynchus mykiss* (Roell *et al.* 1986, Holm *et al.* 1990, Trzebiatowski *et al.* 1981) and estuarine grouper *Epinephelus salmoides* (Teng and Chua 1978, Chua and Teng 1979). The inverse relationship between density and growth rate for *O. mykiss* was attributed to the aggressive, competitive feeding behaviour of dominant individuals (Holm *et al.* 1990). A more frequent feeding schedule reduced but did not remove the depressive effects of high density culture upon growth rate. However, other behavioural aspects of *O. mykiss* may also influence this relationship. Soderberg and Krise (1986) cite earlier research indicating that the acceptable densities for the intensive culture of *O. mykiss* increase with increasing fish size. The current study suggests a similar relationship for the high density culture of *L. calcarifer* > 60 g body weight (Fig. 8.2). Additional studies are required to reveal further information upon this aspect of juvenile culture.

In the present study, the behavioural factors responsible for the reduced growth rates of juvenile *L. calcarifer* of < 50 to 60 g at high densities are uncertain. General observations revealed that small fish formed closely packed schools, often located in the corners of cages while larger fish used the cage volume more effectively. It is possible that small fish within the centre of large schools in high density cages may be exposed to lower oxygen levels and higher localised concentrations of metabolites. These agents could have resulted in reduced growth by increasing the energy demands for maintenance through elevated respiratory activity and increased movement, as fish exchanged relative positions within the school. Webb (1978) reported that increased levels of metabolites and low dissolved oxygen levels can decrease growth rates by reducing appetite and ration intake. An improved dispersion of larger fish within cages may have reduced some of the stress effects at high densities.

The observed reductions in the growth of young *L. calcarifer* at high densities during the current study are of economic importance. Wohlfarth and Moav (1972) have shown how such relatively small differences in the individual weight of young fish can become magnified many times during the ensuing growout period to result in substantially, decreased weight gains. In a previous study (this report, Chapter 9), we have shown that a small difference of 1.2 g in mean start weights of juvenile barramundi of 14.7 and 15.9 g was calculated to result in a difference of 34.7 g in final weights over a culture period of 189 days. These differences in early growth rates gain additional significance in a seasonally varying environment. McKinnon (1990) reviewed the seasonal pattern of growth of *L. calcarifer* resulting from the annual, cyclical change of water temperatures in north-eastern Australia. The most rapid growth occurred at high summer temperature following spawning. However, our study indicates that the greatest reduction in growth rates by high density culture

may coincide with this annual peak of water temperatures, most favourable for juvenile growth during the post-spawning to autumn period. A reduced growth rate at this early stage may unnecessarily prolong the subsequent growout period and increase all associated production costs, particularly for fish spawned during late summer.

Growth rates and seasonal production may be maximised by a number of management practices. The current findings suggest the culture densities of 180 fish/m^3 and 360 fish/m^3 may be appropriate to maximise the growth of juvenile fish of average and good quality respectively. Alternatively, small growout ponds may offer some distinct advantages for juvenile production at lower densities. However, high cage densities of 360 fish/m^3 may be suitable for the growth of larger fish > 60 g in weight to market size, providing adequate water quality can be maintained. If some producers consider it undesirable to distinguish between fish of different quality within each spawning, than an alternative approach based upon total production may be considered. Sakaras (1987) reported significant increases in total production for each culture density between 77 and 231 fish/m^3. The current results suggest that the production of fish cultured at 360 fish/m^3 was significantly greater than either 120 or 180 fish/m^3 but not 240 fish/m^3. Therefore maximum densities of 240 to 360 fish/m^3 for all fish may be suggested from a production viewpoint, but this does not consider the effects of density upon fish of differing quality.

In conclusion, other avenues of research may exist for increasing cage production and decreasing the effects of density associated factors. Increased production of the estuarine grouper, *E. salmoides* was achieved through the consideration of species behaviour in the development of intensive culture methods. Teng and Chua (1979) increased the carrying capacity of cages to 156 fish/m^3 by the inclusion of hides and simultaneously reduced the previously documented depression of growth rates associated with the lower culture density of 120 fish/m^3 (Teng and Chua 1978). Similarly, it may be possible that circular cages or the presence of hides or shelters within cages may result in a better dispersion of juvenile *L. calcarifer*, to reduce overall stress levels and associated growth retardation at high densities. More frequent feeding schedules may also result in improved growth rates at high densities.

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Chapter 9

COMPARISON OF GROWTH, CONDITION AND MORTALITY BETWEEN STOCKS OF BARRAMUNDI *Lates calcarifer*: 1. CAIRNS AND BURRUM RIVER STRAINS

Authors: L.J. Rodgers and J.P. Bloomfield

ABSTRACT

Research has identified multiple, discrete strains of barramundi (*Lates calcarifer*) within Queensland waters. These may be uniquely adapted to their local environment, and thus individual strains may also display different advantages or disadvantages for intensive culture at different geographic sites. Consequently, the current trials were undertaken to compare the simultaneous changes in growth, mortality and condition (K) of tropical and sub-tropical barramundi strains when intensively cultured under identical field conditions. The trials were conducted in a freshwater pond during the autumn to summer period of 1990 to 1991. Both Cairns (16°45') and Burrum River (26°00') strains displayed similar growth rates over a wide range of temperatures and initial culture densities, which varied between 200 and 500 fish/m^3. However, sub-tropical Burrum River fish appeared to have a greater tolerance than Cairns fish to sudden decreases of minimum temperatures to between 15 and 19°C during late autumn and early winter. Burrum River fish also displayed a greater appetite and maintained a significantly higher ($P < 0.05$) condition factor (K) than Cairns fish during early winter to result in a comparatively delayed overwinter mortality. It was proposed that Burrum River fish may have a higher standard metabolic rate than Cairns fish, which affords a small thermal advantage as temperature decreased. However, overwinter mortality began in both strains when average daily temperatures fell below 20°C and cumulative mortality rates of 32 and 39% were recorded for Cairns and Burrum River fish respectively. Both strains gained maximum condition values when growth decreased as daily temperatures varied between 19 and 22.5°C during late autumn and again at similar temperatures before growth increased in early spring. However, both strains exhibited different patterns of mortality in response to a range of bacterial and fungal pathogens encountered during the trials. Trial observations and environmental data from the native habitats of both strains suggested a number of strategies which may influence overwinter survival within the different wild populations.

INTRODUCTION

The barramundi or sea bass, *Lates calcarifer* (Bloch) is a catadromous fish occurring throughout the Indo-West Pacific region. Although predominantly a tropical species, *L. calcarifer* also inhabits the cooler, subtropical waters of central and southern Queensland (Australia) to a latitude of approximately 26°00'S (Grey 1987).

Recent investigations indicate that these tropical and subtropical Australian environments are inhabited by multiple, discrete strains of *L. calcarifer* (Shaklee and Salini 1985, Salini and Shaklee 1987). Evolutionary theory and research on stocks of other species suggests that these individual populations of barramundi may represent a high degree of genotypic adaptation to local environmental conditions (Isley *et al.* 1987, Tave 1990, Moav and Wohlfarth 1975). For instance Jensen and Johnson (1986) investigated genetically discrete wild stocks of Atlantic salmon, *Salmo salar*, and reported that the lower temperature limit for growth varied between stocks according to local environmental temperature regimes.

Similarly, Conover (1990) has also suggested that higher growth rates of some fish species may be achieved by transplanting high latitude genotypes into low latitude environments. The underlying hypothesis of countergradient variation proposed that the basal metabolic rate and the genetic capacity for growth of many poikilotherms, may vary inversely with the length of the growing season across a latitudinal gradient (Conover 1990).

During 1989, anecdotal evidence supporting similar possible adaptive attributes of these different barramundi strains arose from observations of cultured fish near Cairns (17°00'S) in North Queensland. Translocated *L. calcarifer* from Burrum River (26°00'S) appeared to be more cold tolerant, exhibiting better feeding and growth during winter, than fish of the local Cairns strain (16°45'S) that were simultaneously cultured in adjacent cages.

Therefore, the individual Australian strains of *L. calcarifer* may represent a unique and invaluable genetic pool of considerable, current and future significance to the fishing and aquaculture industries based upon them.

Consequently, we conducted a series of investigations based upon the seasonal comparison of growth rates, length/weight relationships, condition (K) factor and mortality syndromes of three geographically distinct Australian strains of *L. calcarifer* cultured in adjacent cages at Walkamin (17°08'S). Burrum River and Cairns strains were compared during the autumn, winter and spring of 1990 in trials 1 and 2. Correspondingly, Weipa (12°40'S) and Cairns strains were similarly compared during the autumn, winter, spring and summer of 1991 to 1992.

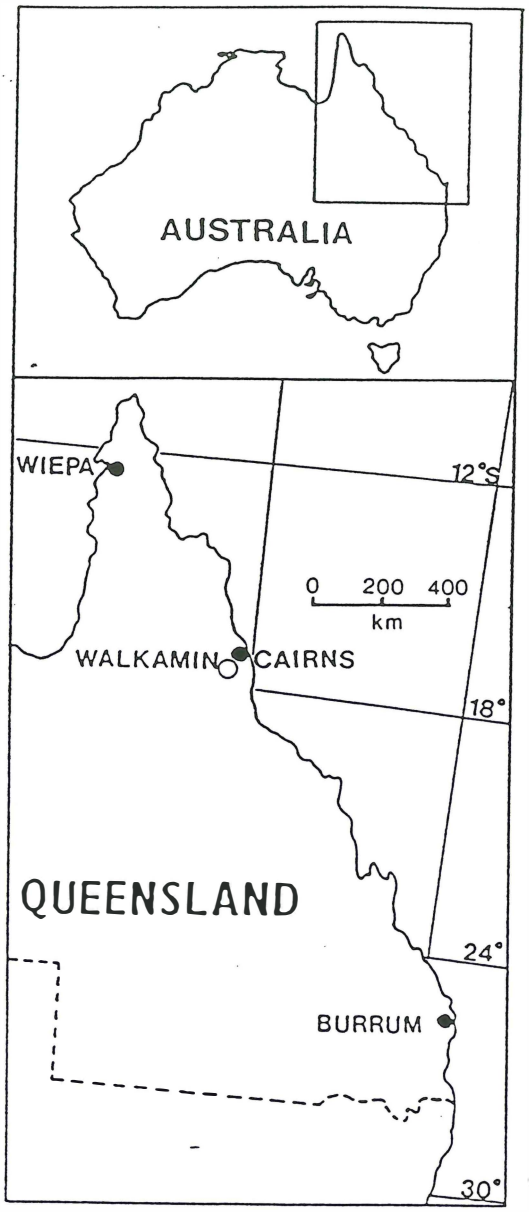

Figure 9.1. Geographic sites of origin for experimental strains of *L. calcarifer* (●)

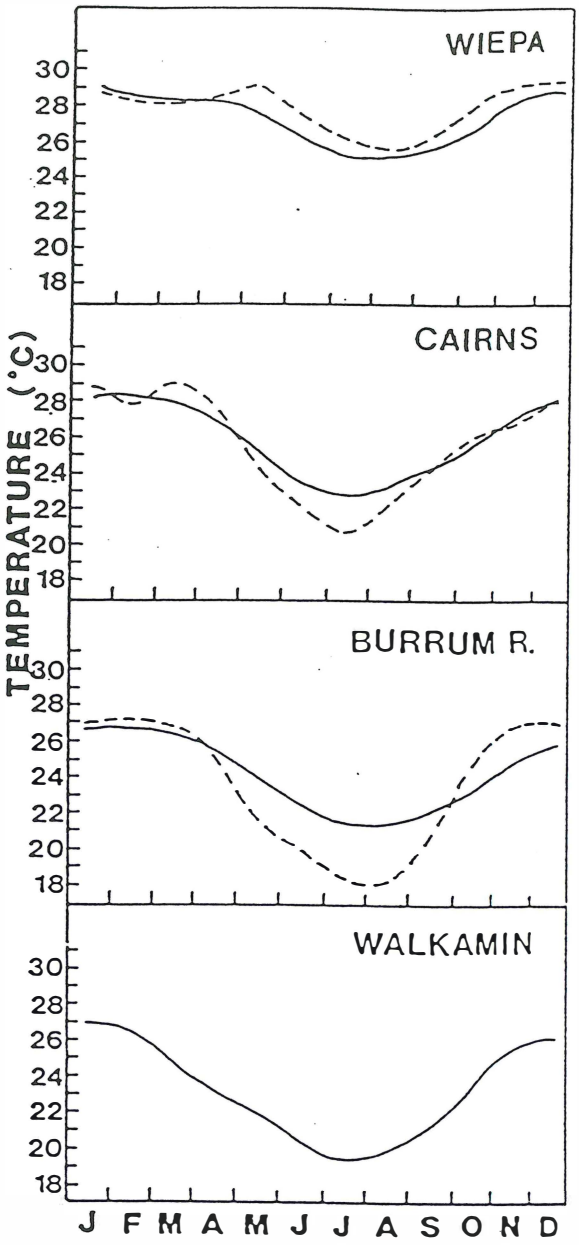

Figure 9.2. Annual profile of average monthly temperature (°C) for near coastal (___) and lower estuarine (---) sites at Weipa, Cairns, and Burrum River. The average monthly pond temperatures at the Walkamin experimental site are also displayed.

METHODS AND MATERIALS

General

The sites of origin for the broodstock used in these investigations are depicted in Figure 9.1, while annual temperature profiles for coastal marine and lower estuarine waters are presented in Figure 9.2. The coastal temperature data for Weipa, Cairns and Burrum sites were based on 16, 32 and 37 years respectively of averaged monthly temperatures derived from the "Ships at Sea Temperature program" (Lough, Australian Institute of Marine Science pers. comm. 1991). Lower estuarine temperatures were compiled from four years of research data for the Weipa site (CSIRO unpublished data), and approximately two years for Cairns (J. O'Brien, NFC, Cairns, pers. comm. 1992) and south Queensland sites (C. Lupton, Burnett Heads Fisheries Centre and J. Burke, Southern Fisheries Centre, pers. comm. 1991, 1992). Temperature data for Walkamin is based upon four years of data which includes the current period of study.

Pond temperatures at the Walkamin experimental site were recorded daily during the study, using minimum/maximum thermometers located at a depth of 90 cm within cages. Seasonal trends in maximum and minimum experimental temperatures are shown in the results of trials as moving averages in which the temperature readings shown for any single day, were averaged with the corresponding data of the immediately preceding and subsequent days. This data is presented for descriptive purposes but real temperature values have been used throughout the results and discussion, in reference to observations of feeding behaviour and mortality.

Each experimental block used throughout all trials consisted of a cage module of 1.00 m^3 volume and 1.38 m^2 surface area, with a centre partition dividing each into two cages of equal size. Each 0.5 m^3 cage was stocked with a different strain of *L. calcarifer* and aerated with a single airstone.

Initially the cages were constructed of 6 mm mesh but the mesh size was increased to 10 mm to prevent accumulations of excess feed which resulted from reduced appetite during late autumn. All cage modules were supported by floating PVC collars and randomly positioned about a floating pontoon within a 0.12 ha freshwater pond. The cages were shifted to a new experimental pond at approximately 100 day intervals to maintain a high quality culture environment.

Handling injuries were minimised by synchronising the interpond transfer with the measurement and salt bath procedures, which were repeated at the end of each 21 day trial period. Individual fish were measured to the nearest mm (TL) and weighed to the nearest 0.1 g.

A sample of eight thousand juvenile *L. calcarifer* of approximately 12 mm total length (TL) were obtained from a saltwater hatchery following a mid-January, induced spawning of local captive Cairns broodstock. A similar sample of juvenile

L. calcarifer was also obtained from another hatchery following a spawning of captive Burrum River broodstock on the 5th January, 1990.

Both groups of fish were acclimated to freshwater and transferred to the Walkamin Research Station during early February where they were ongrown utilising freshwater zooplankton. Subsequently, juvenile barramundi of 20 to 30 mm TL were weaned onto a dry diet and further ongrown in cages located within a freshwater pond until required for experimental purposes.

Mortalities were recorded daily together with pH, maximum and minimum temperatures, dissolved oxygen and general observations of feeding behaviour. Condition (K) was estimated using the formula $K=\frac{W(gms)}{L^3(cms)}x100$ while specific growth rates were calculated using the formula: $G_w=(\frac{\log_e W_2-\log_e W_1}{t(days)})x100$ Elliot (1975) where w_1 = mean individual weight at the beginning of each trial period and w_2 = mean individual weight at the end of each trial period.

Statistical methods for ANOVA and regression analyses were performed according to Zar (1984).

Trial 1.

Trial 1 commenced on 14 May, 1990 and compared the growth, mortality and condition (K) of Cairns and southern Burrum River fish for a culture period of 63 days during autumn and early winter.

One cage of 0.5 m^3 within each of 4 experimental blocks was allotted to each strain and individually stocked with 250 fish, prior to an acclamation period of two weeks. Juvenile *L. calcarifer* of Cairns and Burrum River fish measured 66.2 $\pm$ 4.7 mm. in total length (T.L), (4.1 $\pm$ 0.8g) and 65.1 $\pm$ 3.3 mm. T.L. (3.7 $\pm$ 0.5g) respectively at the start of the trial. A sample of 50 fish was taken from each cage at 21 day intervals, measured to the nearest mm and weighed to the nearest 0.1g. After weighing and measuring, the sample and all of the remaining fish from each cage were given a salt bath of 10% for 30 minutes while the cages were cleaned with a water jet.

A commercially available dry diet[1] was offered to fish four times daily between 8.00 am and 5.00 pm at a constant rate of 4.5% of body weight per day throughout the trial. Feed quantities were revised at the conclusion of each 21 day trial period, based upon calculated mean individual weights and cage density.

1 Barramundi Starter Diet manufactured by Aquafeed Products, Australia to the specifications of formulae no. 521007 proximate composition as follows : crude protein 50%; lipid 10%; carbohydrate 15.3%; ash 12.7%; fibre 3%; and moisture 8%. The use of this product for research purposes does not imply endorsement of the company or its products.

Trial 2.

This trial began in mid July during the early winter of 1990 and continued until the end of January during mid summer. The purpose of the trial was to continue the comparison of Cairns and Burrum River fish through winter, spring and summer but at lower cage densities than used during trial 1. The experimental protocol of this study was similar to that of trial 1, but on this occasion one hundred fish of each strain were stocked separately into 5 replicate cages of 0.5 m^3 volume.

Juvenile *L. calcarifer* of the Cairns strain initially measured 105.8 $\pm$ 8.7 mm (mean $\pm$ s.d.) in total length, (TL) and 15.9 $\pm$ 4.3 g in weight while Burrum River fish measured 102.9 $\pm$ 8.1 mm (TL) and 14.7 $\pm$ 3.5 g in weight at the start of the trial. Samples of 35 fish were taken from each cage at the conclusion of each trial period of 21 days, prior to being weighed to the nearest 0.1g and measured to the nearest mm (TL). The trial continued for nine consecutive trial periods.

RESULTS

Trial 1

Temperature and Feeding Behaviour

The steady decline of daily minimum and maximum water temperatures during the trial is presented in Figure 9.3a together with the average daily temperatures which decreased from 22°C during period 1 to 20.4°C and 19.2°C during trial periods 2 and 3 respectively. Minimum temperatures of $\leq$ 19°C occurred during days 30 to 35 and reduced feeding was subsequently observed with the further, rapid decline of minimum temperatures to $\leq$ 17°C for a short duration (Fig. 9.3a). The return of warmer temperatures at day 42 was accompanied by improved feeding behaviour.

However, a sustained depression of minimum temperatures of below 16°C occurred during days 47 to 55 of trial period 3 and very reduced feeding was again observed. Although Cairns strain fish responded to feeding by slowly swimming through the sinking pellēts, it became apparent afterwards that their appetite was comparatively reduced at these low temperatures. Further examination revealed that uneaten feed pellets were accumulating in all of the replicate cages of Cairns strain fish, while the full morning ration was still being consumed in the cages containing Burrum River strain fish.

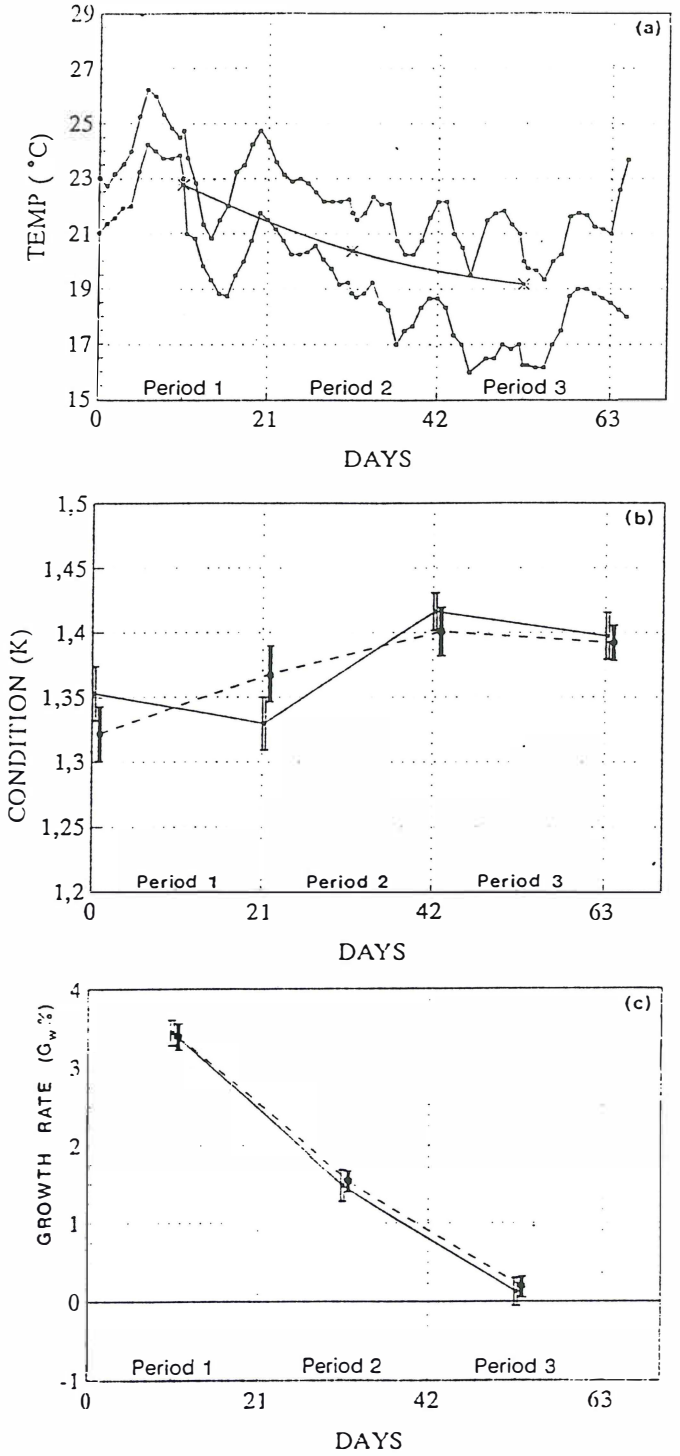

Figure 9.3. Minimum (■), Maximum (■) and averaged (*) daily temperatures (°C) for each trial period during trial 1, (a); Condition factor (K) for Cairns (___) and Burrum River (---) strains during each trial period of trial 1, (b); Specific growth rate (% weight gain day^{-1}) for Cairns (___) and Burrum River (---) strains during trial 1, (c) (mean ± S.E.).

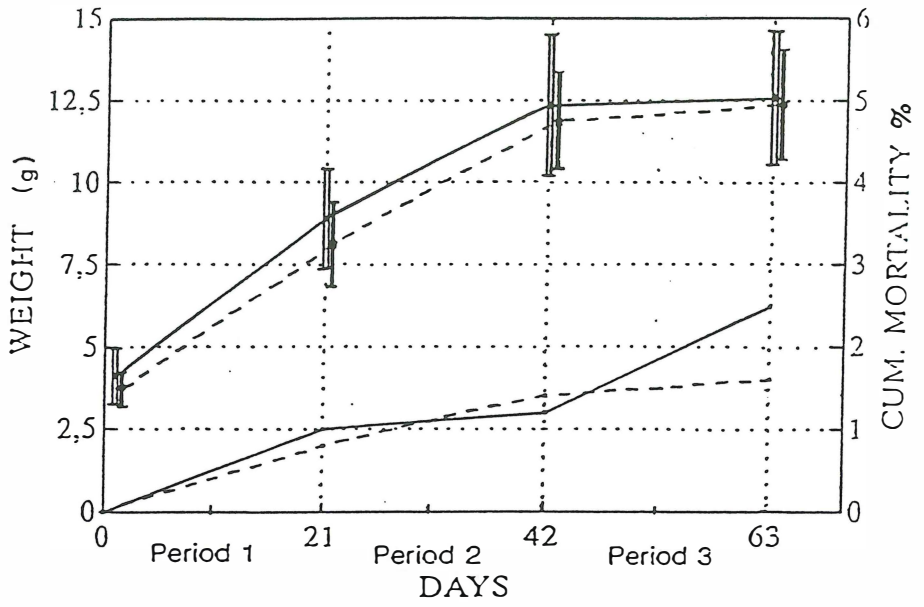

Figure 9.4. Cumulative mortality (%) and Weight (g) of Cairns (___) and Burrum River (---) strains during each period of trial 1 (mean $\pm$ SE).

Both strains initially displayed more active feeding behaviour at warmer afternoon temperatures. However, this activity also decreased in intensity for both strains as the cold spell continued. Daily minimum temperatures of $\leq$17°C were recorded for eleven consecutive days during growth period 3 (Fig. 9.3a) and eventually both strains became increasingly lethargic.

The appetite and locomotory activity of both strains improved after day 57 with return of increased temperatures, which continued until well after the trial conclusion.

Condition (K)

Both strains gained condition (K) during the first two trial periods but maintained similar mean values of approximately 1.4 during trial period 3 (Fig 9.3b), when cooler minimum temperatures prevailed (Fig 9.3a). There was no significant difference in condition ($P > 0.05$) between strains during the trial.

Growth (Gw)

Cairns and Burrum barramundi strains displayed similar rapidly declining growth rates during days 0 to 42 when average daily temperatures decreased from 22.7°C to 20.4°C (Fig 9.3a, c). The further decline in temperature to 19.2°C during

period 3 was accompanied by the almost complete cessation of growth in both strains. No significant differences in growth rates ($P > 0.05$) were observed between strains during the study.

Mortality

Similar low level mortalities occurred within both strains during the first two trial periods. However, Cairns fish appeared to display an increased mortality due to cold exposure during the final trial period 3 (Fig 9.4). Thirteen Cairns fish and two Burrum River fish died during this final trial period (Fig. 9.4). Dying fish exhibited epidermal lesions characterised by the presence of *Saprolegnia sp.* fungus. Mortalities abated rapidly during and after the trial conclusion as warmer minimum temperatures prevailed.

RESULTS

Trial 2
Temperature and Feeding

Both strains exhibited satisfactory feeding behaviour which decreased with the falling temperatures of days 1 to 10 (Fig. 9.5a). Subsequent minimum temperatures of approximately 15 to 16°C were associated with very poor feeding behaviour and the occurrence of mortalities, particularly for Cairns strain fish (Fig. 9.5c). Feeding activity temporarily resumed in both strains as minimum temperatures increased to > 20°C at the end of trial period 1.

Minimum temperature declined rapidly again to between 16°C and 18°C during days 22 and 40 of period 2. All fish appeared lethargic and displayed little response to feeding, at minimum temperatures below 17°C during day 23 to 30.

However, feeding activity increased during trial period 3 with the return of warmer minimum temperatures of between 18 and 19.5°C during days 43 to 63 (Fig. 9.5a). The ensuing springtime increase in temperature was accompanied by very active feeding behaviour which continued throughout the remainder of the trial.

Condition (k)

Burrum River barramundi displayed significantly greater condition values ($P < 0.05$) than Cairns fish at the conclusion of trial period 1 (Fig 9.6a). Subsequently, the condition of Cairns and Burrum River fish decreased and there were no significant differences in condition between strains at the conclusion of period 2.

Feeding activity returned with the warmer minimum temperatures of between 18 and 19.5°C during days 43 to 63 (Fig. 9.5a) and the condition of fish in all cages

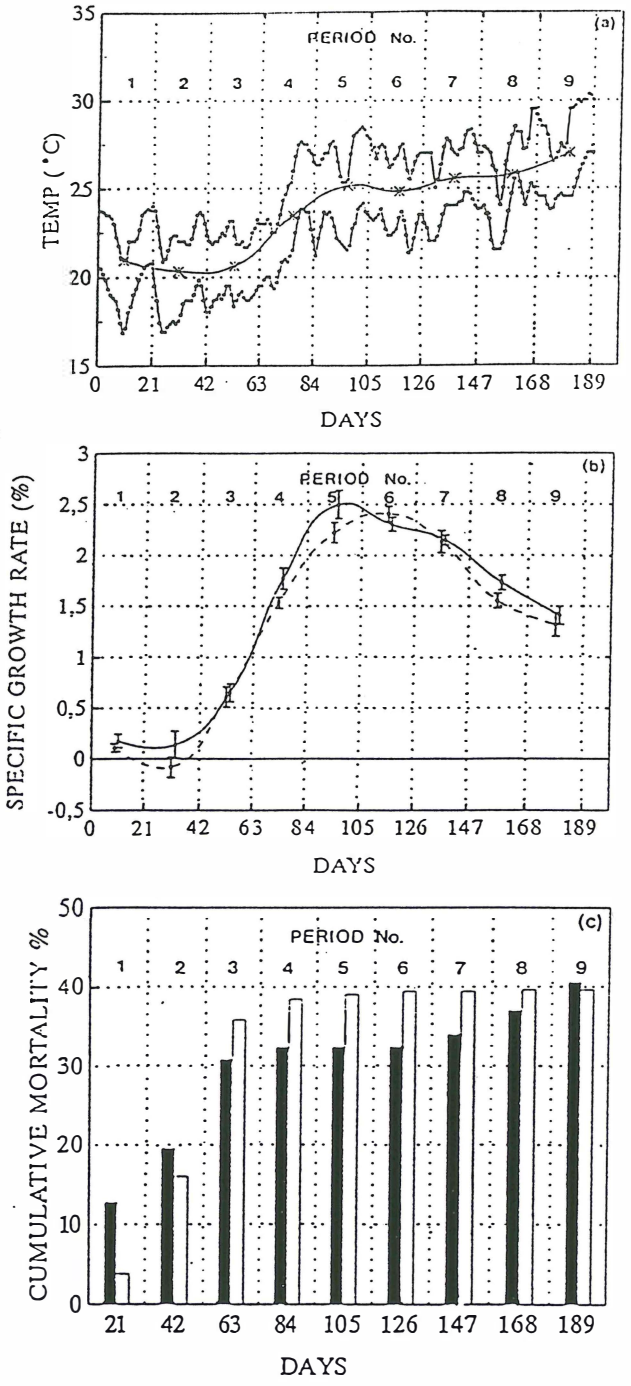

Figure 9.5. Minimum (■), Maximum (■) and averaged (*) daily temperatures (°C) for each trial period during trial 2, (a); Specific growth rates (% weight gain day^{-1}) for Cairns (___) and Burrum River (---) strains of *L. calcarifer* during trial 2 (Mean $\pm$ SE), (b); Cumulative mortality (%) for Cairns (___) and Burrum River (---) strains during each trial period of 21 days during of trial 2, (c).

increased to peak values (Fig. 9.6a). An average daily temperature of 20.6°C prevailed and Cairns fish displayed significantly greater condition ($P<0.01$) than Burrum River fish during this third trial period (Fig 9.6 a).

Both strains initially lost condition during trial periods 4 and 5 with the rapid onset of growth during spring but gained condition during period 6 as temperatures stabilised. However the condition values of all fish slowly decreased with increasing fish size and Cairns fish maintained significantly greater condition ($P<0.01$) than Burrum fish during periods 4 to 9 (Fig 9.6a).

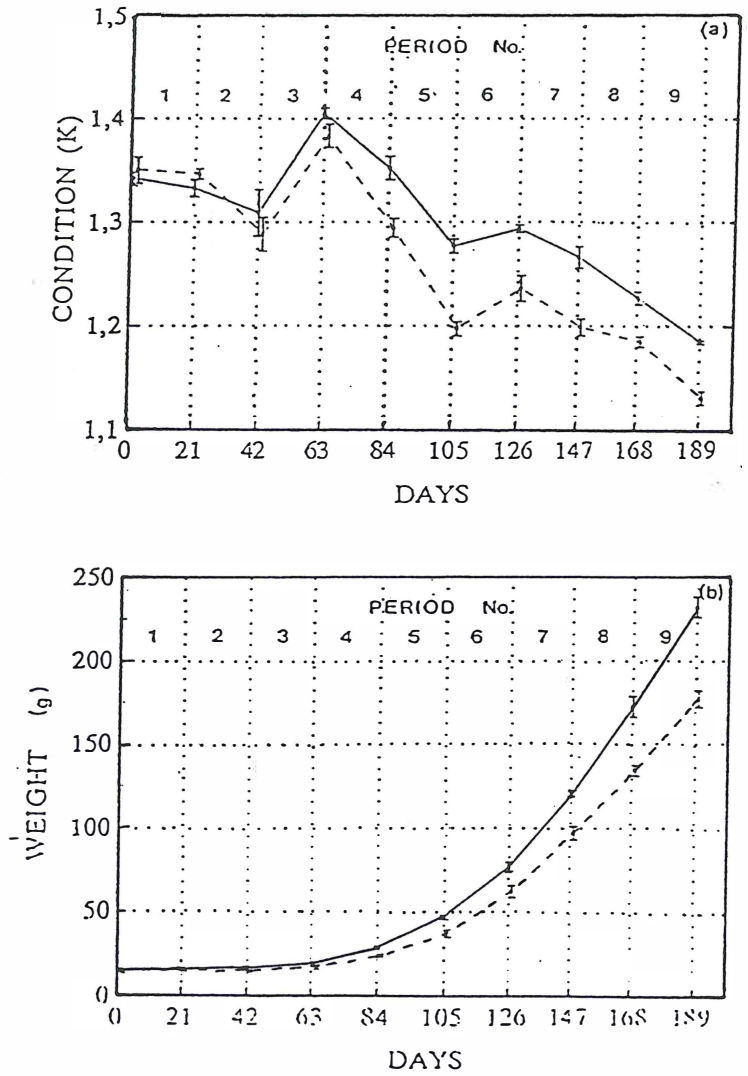

Figure 9.6. Condition factor (K) of Cairns (___) and Burrum river (---) fish during trial 2, (a); Cumulative weight of Cairns (___) and Burrum River (---) strains during each period of trial 2 (Mean $\pm$ SE).

A differential length/weight relationship developed for each strain as individual size increased (Fig. 9.6b). Regressions were performed to describe the length/weight relationships of both barramundi strains during growth periods 4 to 9.

Cairns Strain log Wt = 2.939 * log TL - 4.756 (R^2 = 0.99)
Burrum River Strain log Wt = 2.849 * log TL - 4.579 (R^2 = 0.99)
(Wt is the weight in g and TL is the total length in mm).

A comparison of slopes revealed that these regressions were significantly different ($P < 0.001$).

Specific growth rates (Gw)

Both barramundi strains exhibited similar low, mean winter dasily growth rates of below 0.25% during trial periods 1 and 2 (Fig. 9.5b). Four cages of Burrum River fish displayed negative growth during trial period 2 when an average daily temperature of 20.3°C was present.

Cairns and Burrum fish responded to rising spring temperatures with similarly increased daily growth rates of 0.6 and 0.7% respectively during period 3. Temperatures continued to rise and an average daily temperature of 23.5°C was subsequently recorded during period 4 when the mean daily specific growth rates of both strains further increased to between 1.5 and 1.75%. Cairns fish displayed a significantly greater growth rate ($P < 0.05$) than Burrum River fish during this period (Fig. 9.5b).

The specific growth rates of Cairns and Burrum River fish reached maximum values during growth periods 5 and 6 respectively, as average daily temperatures increased and stabilised at approximately 25°C (Fig. 9.5a). Significantly greater specific growth rates ($P < 0.05$) were recorded for Cairns fish during growth period 5 (Fig. 9.5b). However, Burrum River fish displayed significantly greater growth rates ($P < 0.01$) than Cairns fish during growth period 6 when minimum temperatures decreased to approximately 21°C during a sudden, unseasonal cold spell (Figs. 9.5b).

Average daily temperatures slowly increased to 27°C during periods 7,8 and 9 and there were no further significant differences in specific growth rates between strains (Fig. 9.5b).

The mean weight of Cairns fish (231.5g) was 54.8 g greater than Burrum River fish when the trial concluded during early summer (Fig 9.6b).

Mortality

The cumulative mortality of Cairns fish increased rapidly during trial period 1 when low minimum temperatures of 15 to 16°C were present (Fig 9.5c). Burrum River fish displayed a lower mortality during this initial period which increased during period 2. However, cumulative mortality continued to rise for both strains during period 3.

A two way ANOVA of transformed mortality data indicated significant interaction ($P < 0.005$) between strains and trial periods as winter progressed. Cairns fish suffered higher initial mortalities while Burrum River fish displayed a trend of delayed mortality which increased with winter duration (Fig. 9.5c). Cumulative overwinter mortality rates of 32 and 39% were recorded for Cairns and Burrum River fish respectively.

Minimal mortalities of both strains occurred during trial periods 4, 5 and 6 as minimum temperatures increased to >20°C. However, mortality subsequently increased in all replicate cages of Cairns fish during days 126 to 189 of periods 7, 8 and 9. (Fig 9.5c, Table 9.1).

Table 9.1.

Mortality of Cairns strain *L. calcarifer* over final days of Trial 2.

Replicate	Mortality		
	Period 7	**Period 8**	**Period 9**
1	1	5	6
2	2	0	2
3	1	4	1
4	1	3	7
5	3	3	2
TOTAL:	8	15	18

Symptoms associated with this mortality included a swollen abdomen and peritonitis. *Aeromonas hydrophila, A. sorbria,* and *Pasteurella shigelloides* were identified from blood and kidney isolates, while *Proteus vulgaris* was additionally identified from abdominal isolates (I. Anderson, Oonoonba Veterinary Laboratory, pers. comm., 1991). Abdominal adhesions between visceral organs and parietal peritoneum together with large bacteria filled omental abscesses were also present in some infected fish.

Burrum River fish in cages immediately adjacent to Cairns fish did not display similar symptoms or mortalities during this period.

Routine water quality analyses at the time of infections revealed insignificant levels of total ammonia nitrogen and nitrite throughout this disease outbreak. The subsequent addition of oxytetracycline to the diet at 1 mg/kg dry diet/day resulted in a rapid reduction of mortality after the trial conclusion.

DISCUSSION

The two strains of *L. calcarifer* compared in these trials, originated from native habitats displaying different thermal regimes (Figs. 9.1 & 9.2). The Cairns strain inhabits tropical waters (approximately 15°30' to 22°30'S) while the Burrum River strain inhabits cooler, sub-tropical to warm temperate waters (approximately 22°30' to 26°00'S). Mortalities of wild barramundi populations have been reported during cold spells in latitudes ≥23°26'S (Mackinnon, 1989). Therefore, the duration and severity of low temperatures during winter appears to influence barramundi survival in wild populations at more southern latitudes.

Burke has investigated the temperature tolerance of these strains of *L. calcarifer* in laboratory trials which utilised small fry of 11 to 12 mm total length. Both strains displayed a similar preferred temperature of 27 to 36°C and a maximum thermal limit of 42°C. The final cold death points (T_{100}) were approximately 12°C for both strains although significantly different ($P < 0.05$) T_{50} values of 14.62°C and 13.78°C were recorded for Burrum River and Cairns strains respectively (J. Burke, SFC pers comm., 1992). These results suggest that the low latitude Cairns strain, which originated from a warmer thermal regime, was more cold tolerant. In contrast, Tave (1990) reported that, *Tilapia nilotica* which originated from approximately 32°00'N, 10°00' and 7°30'N, exhibited corresponding mean lethal temperatures of 50°F, 54°F and 57°F respectively, which decreased with increasing latitude.

However, the current trials suggest that more subtle differences may exist between strains with regard to their tolerance of low winter temperatures under field conditions. Burrum River fish consumed a greater proportion of daily ration when sustained minimum temperatures of 16.0°C to 17.5°C occurred during late autumn and early winter of trial 1. Significantly greater condition values ($P < 0.05$) were confirmed for this strain during early winter at the conclusion of the first growth period of trial 2 (Fig. 9.5a). These observations and mortality patterns suggest that the capacity of Burrum River fish to maintain appetite and condition for a longer period during early winter may have been associated with the comparatively delayed onset of significant mortality within this strain during mid to late winter (Fig. 9.5c). In comparison, Cairns fish exhibited reduced appetite, decreasing condition and

marginally lower growth rates prior to the onset of rapidly increasing mortality in association with the cold spells of autumn and early winter (Figs. 9.3c, 9.4, 9.5c & 9.6a). An average daily temperature of 20.4°C during period 3 coincided with increased mortality of Cairns fish in trial 1, while a corresponding temperature of 19.2°C was associated with the onset of increased mortality of this strain during period 1 of trial 2 (Figs. 9.4 & 9.5c). Minimum temperatures of >20°C during spring coincided with the conclusion of overwinter mortality.

Cheong (1989) and Kungvankij (1984) associated the mortality of cultured *L. calcarifer*, originating from low latitude strains, with prolonged periods of winter temperatures below 20°C. Wu (1989) has also reported mortalities of cage cultured *L. calcarifer* in the Hong Kong region when temperatures fell to 15°C for periods of > 7 days. Results of the current study indicate that the effects of low, average temperatures may be exacerbated by the protracted duration of winter, as evidenced by increasing mortality and the presence of a cold torpor in both strains during mid and late winter (Figs. 9.5c). In addition, the difference in appetite and condition evident between strains during the early winter in trial 2 disappeared as winter progressed, even though similar average daily temperatures below 21°C prevailed. (Fig. 9.5a).

These results suggest an ongoing physiological adjustment by fish during winter, as appetite was reduced and energy stores were mobilised with continued exposure to sub-optimal temperatures. It is possible that the greater initial appetite but similar growth rates of Burrum River fish, together with the delayed mortality and greater condition of Burrum River fish during early and mid-winter reflect a higher metabolic rate, as has been proposed for higher latitude conspecifics (Conover 1990, Cossins and Bowler 1987). The occurrence of negative growth rates in four replicates of Burrum River fish during period 2 of trial 2, and the subsequent simultaneous increase of growth rates to similar levels as displayed by Cairns fish during days 42 to 63 (Fig 9.5b) of early spring, also supports the concept of a higher metabolic rate for Burrum River fish. In addition, the removal of block effects for specific growth rates during period 6 of trial 2 revealed significantly greater growth rates ($P < 0.01$) for Burrum River fish when an unseasonal cold spell suddenly reduced minimum temperatures to below 22°C during mid spring. Cairns fish displayed a corresponding decrease in growth rates at this time. This greater degree of functional independence of metabolism from rapid temperature depressions for Burrum River fish may also suggest a higher metabolic rate.

Several observations during this study may relate to increased mortalities within wild barramundi populations during winter periods. Cossins and Bowler (1987) indicated that a period of cold torpor, as observed in this study, corresponded to a period of increased vulnerability to predation. Increased winter mortality may also result from a decreased physiological state of a species and its inability to respond

adequately to pathogens at low or decreasing temperatures (Wedermeyer 1970, Ellis 1981). Consequently, a significant prerequisite for the survival of a tropical species such as *L. calcarifer* near the geographic limits of its distribution, is the ability to maintain metabolism at a level sufficient to permit a rapid response to pathogens at low temperatures. In this respect, Burrum River barramundi exhibited delayed susceptibility to skin infections by fungus, *Saprolegnia sp* during early winter, in comparison to the Cairns strain. In addition, the bacterial peritonitis disease syndrome of late spring and summer was confined to the Cairns strain even though Burrum River fish were located in adjacent cages (Table 9.1). The strains appear to differ in their resistance to the range of pathogens encountered during these trials.

These investigations also revealed some similarities between the two strains of *L. calcarifer*. The highest condition values were recorded for both strains during autumn as growth decreased and daily temperatures ranged from approximately 19°C to 22.5°C (Figs. 9.3b and 9.6a). A similar relationship of increased condition due to enhanced lipid storage with decreasing temperatures was reported for large mouth bass *Micropterus salmoides* (Nimi and Beamish, 1974). The condition factors of both strains increased to similar high values during late winter and early spring of period 3 (trial 2), as the growth of both strains also resumed when a temperature range of approximately 19 to 22.5°C was present (Fig. 9.6a). The subsequent decreasing condition values of both barramundi strains at lower temperatures appeared to be linked with decreased appetite, particularly evident during morning feeds when lower minimum temperatures prevailed (Figs. 9.5a & b). The mortality of both strains decreased simultaneously during period 4 of trial 2 following peak condition values (Fig. 9.5c). In addition, the condition factor for both strains decreased during periods 4 to 9 of trial 2 with the diversion of energy into rapid growth as temperatures increased.

The specific growth rates (G_w) of both strains were similar over a wide range of temperatures throughout these investigations (Figs 9.3c and 9.5b). However, Burrum River fish exhibited significantly greater growth rate during growth period 6 of trial 2 when a temporary decrease in the growth rate of Cairns fish coincided with a series of short but rapid decreases of minimum temperatures to approx 21.0°C (Figs. 9.5a & b). The different culture densities of 200 and 500 fish/m^3 during trials 1 and 2 did not result in any apparent differences in growth rates between strains at similar temperature ranges.

Our results did not demonstrate a significant increase in specific growth rate with increasing latitude as proposed by Conover (1990), although an increased metabolic rate was suggested. However, Burrum River fish may have shown improved growth when compared with Cairns fish, if fluctuating minimum temperatures between 17 and 20°C had prevailed for a longer period during autumn and early winter. The observed difference in mean weights at the conclusion of trial 2 was

principally attributed to the small difference in the start weights between the two strains as Burrum River fish displayed a mean start weight that was 1.2 g lower than Cairns fish at the beginning of trial 2 (Fig 9.6b). Although, the mean weight Cairns fish was 54.7 g greater than Burrum River fish at the trial conclusion, the mathematical substitution of the slightly lower mean start weight of Burrum River fish with the subsequently observed growth rates of Cairns fish reduced the final difference between strains to 20 g. Therefore, the observed difference in final mean weights between strains was principally attributed to a magnification effect of the small initial difference in start weights (Moav and Wohlfarth 1973).

The results suggest that reduced appetite, increased mortality and decreased growth rate at low minimum temperatures ($\leq$ 19°C) represent a significant problem for both wild stocks and cultured *L. calcarifer* in the subtropical to warm temperate waters of central and southern Queensland. However, Cousins and Bowler (1987) indicated that many species actively select more favourable thermal conditions during times of environmental adversity. In addition, Burke (J. Burke, Southern Fisheries Research Centre, pers comm. 1992) has demonstrated that salinities of 10-15% offer an energy saving, osmotic advantage which decreased the lethal effects of low temperatures upon both Cairns and Burrum River strains of *L. calcarifer*. The temperature regimes displayed in Fig. 9.2. also suggest that a considerable thermal advantage may be afforded by downriver migration to warmer coastal and lower estuarine waters during winter. Anecdotal information suggests that the lethal effects of low temperature and extended winter duration at the most southern latitudes of this species distribution, may indeed be offset by downriver migration. Consequently, mortality within wild populations may be modified by behaviour, particularly in southern habitats.

From an aquaculture perspective, the information derived from trials 1 and 2 may allow the introduction of economies in the cost of overwintering fish. Firstly, feeding practices and feed composition could be modified to accommodate changes in appetite, activity and possibly lipid storage which are associated with depression of minimum temperature to 19°C during autumn and early winter. Secondly, it may also be prudent to pursue culture practices for *L. calcarifer* in more southern latitudes which are based on brackish water or marine culture methods rather than freshwater culture techniques during winter. In addition, stock should not be handled unnecessarily during the mid-winter to late winter period when most vulnerable to external fungal infections.

General observations made during the conduct of these trials suggested that both overwinter growth and mortality of *L. calcarifer* may be size dependant. Similar size dependence of overwinter mortality has been reported for other fish species (Post & Evans, 1989, Oliver *et al* 1979). Consequently, it may be possible to identify the proportion of stock at greatest risk of mortality and simultaneously

reduce feed costs, culture densities and exposure to the pathogens of dying fish, by performing a mid or late autumn cull of smaller individuals. These concepts were investigated further during subsequent trials.

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