ResearchOnline@JCU

JAMES COOK UNIVERSITY AUSTRALIA

This file is part of the following work:

Herbert, Brett (2005) ***Feeding and growth of golden perch (Macquaria ambigua), and assessment of its potential for aquaculture.*** **PhD Thesis, James Cook University.**

Access to this file is available from:

https://doi.org/10.25903/e6dg%2Dwv71

Copyright © 2005 Brett Herbert

The author has certified to JCU that they have made a reasonable effort to gain permission and acknowledge the owners of any third party copyright material included in this document. If you believe that this is not the case, please email researchonline@jcu.edu.au

FEEDING AND GROWTH OF GOLDEN PERCH (*MACQUARIA AMBIGUA*), AND ASSESSMENT OF ITS POTENTIAL FOR AQUACULTURE

Thesis submitted by
Brett Herbert

For the Degree of Doctor of Philosophy in the School of Marine Biology and Aquaculture
James Cook University
September 2005

ELECTRONIC COPY

I, the undersigned, the author of this work, declare that the electronic copy of this thesis provided to the James Cook University Library, is an accurate copy of the print thesis submitted, within the limits of the technology available.

_____ _____

Signature Date

STATEMENT OF ACCESS

I, the undersigned, the author of this thesis, understand that James Cook University will make it available for use within the University Library and, by microfilm or other means, allow access to other users in other approved libraries. All users consulting this thesis will have to sign the following statement:

> In consulting this thesis, I agree not to copy or closely paraphrase it in whole or in part without the written consent of the author; and to make proper public written acknowledgement for any assistance which I have obtained from it.

Beyond this, I do not wish to place any restriction on access to this thesis.

---------- ----------

Brett Herbert

STATEMENT OF SOURCES

DECLARATION

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

---------- ----------

Brett Herbert

ACKNOWLEDGEMENTS

I acknowledge the support and assistance of my colleagues at the Freshwater Fisheries and Aquaculture Centre, Walkamin, for their assistance with sampling and running experiments. In particular, Peter Graham, Neil Harris, Ben Hockings, Dave Bull and Bevan O'Grady assisted with all aspects of day to day running and monitoring of experiments (particularly feeding and water quality monitoring), maintenance of equipment, assistance with measuring and grading fish, and support when things went wrong. I also acknowledge the assistance of DPI&F biometricians Scott Foster, Bob Mayer and Joanne de Faveri for guidance and advice on statistical analysis. Peter Graham is a co-author of the papers due to his dedication and input into experiments, and input into material prepared for publication separately to this thesis. I also thank the veterinary staff at Oonoonba veterinary laboratory for assistance and advice with disease issues, and for providing histological material for me to examine.

This work was conducted as an element of my employment by the Queensland Department of Primary Industries, which provided facilities, infrastructure and administrative support to enable this work to be conducted.

I wish to acknowledge the input of Chris Barlow, my initial co-supervisor who suggested that this study be undertaken. I also acknowledge the input of my supervisory team, Peter Appleford, Paul Southgate, and Rocky de Nys who assisted with commencement and finalisation of the thesis, and provided valuable comments and criticisms of the drafts. Stuart Rowland, Mike Rimmer, Jeff Gooley and Brett Ingram also provided input and suggestions on draft material for publications, and advised on experimental design and direction. Members of the Aquaculture Association of Queensland provided input into the direction of the research (for example focus on weaning fingerlings rather than larvae), and also advised me of requirements for further research when they started farming golden perch.

I also thank my family for their unfailing support during the course of this study.

Abstract

Golden perch (*Macquaria ambigua*) is a valuable freshwater fish native to south eastern Australia. The fishery for this species is diminishing and there exists an opportunity to develop aquaculture techniques for commercial production. Mass production techniques for fingerlings have been developed, but the paradigm that weaning of golden perch onto artificial foods essential for aquaculture development was difficult or impossible, impeded investigations into optimising aquaculture techniques for the species. The aims of this study were to develop aquaculture techniques for golden perch, focussing on three major issues: (1) weaning fingerlings on to artificial foods; (2) the nursery phase of production; and (3) growout of the fish to market size. Additional investigation of a destructive epizootic in aquaculture golden perch was also undertaken to develop control techniques for mixed motile aeromonad/ciliate protozoan infections.

Weaning of fingerling golden perch was investigated after discussions with industry indicated that reliable, cheap, mass production of fingerlings was regularly undertaken and there was no real need to wean larvae or fry. Fingerlings used in weaning experiments (18-31 mm TL, 0.1-0.5 g) are produced by commercial hatcheries for recreational fishery enhancement. A weaning technique using frozen *Artemia* nauplii and formulated crumble food ice blocks was developed, in which the frozen *Artemia* was gradually replaced by crumble food particles. *Artemia* nauplii slurry was replaced with crumble food particles, with the proportion of crumble increasing by ten percent each day until 100% crumble food was being fed to the fingerlings after ten days. Iceblocks of pure *Artemia* slurry, presented in a mesh bag, were fed over an acclimation period to habituate fish. Subsequently prepared co-feeding (*Artemia*/crumble) iceblocks were fed using the same method. This method was successful resulting in 77% weaning success.

Due to the relatively high cost of *Artemia*, frozen zooplankton was tested as an alternative and produced better weaning results than the control *Artemia* weaning treatment in terms of survival (16% better) and growth (1.57 g ± 0.55 g on zooplankton against 1.11 ± 1.07 g on *Artemia*). This was adopted as the control treatment and the method of choice for mass weaning of fingerlings for nursery and

growout trials. After requests from industry for alternatives to plankton or *Artemi*a in weaning fingerlings, commercially available seafood products were tested as co-feeding diets in the weaning process. Squid, fish roe, prawn, mussel, scallop, and fish were tested against a zooplankton control. The seafood materials were processed to be a similar size to the plankton control (500 µm – 1.5 mm) and co-fed with crumble as iceblocks in 10% increasing increments and compared to a zooplankton control. Weaning using zooplankton as a co-feeding diet was far superior to other treatments tested in terms of both survival and growth. The best survival rates of the seafood treatments (the fish roe co-feeding treatment) was 39% and the poorest survival rate of zooplankton co-fed controls was 87% in the first trial. End weights of golden perch on all weaning treatments were significantly different ($P<0.05$) to the control. The olfactory and organoleptic properties of the co-feeding weaning diets were suggested to be of great importance in success of weaning.

Weaning techniques were further refined by investigating the effect of the duration of the transition period, the size of fingerlings, and light intensity on weaning success in fingerling golden perch. Attempts to reduce the co-feeding period or alter the ten-day transition period reduced weaning success significantly, from 62% feeding fish in the control treatment to between 36 and 46% for abbreviated treatments, suggesting that a minimum ten day transition period was essential for golden perch fingerlings. Mean weights at the end of the trial were also significantly different ($P<0.05$) (2.1 ± 0.97 g for controls, and 1.6 ± 0.83 g to 1.8 ± 0.99 g for treatments). The effect of size of fingerlings at weaning was tested (using fingerlings of the same age but different sizes), and no significant differences in weaning success were found between sizes from 0.1–0.24 g, suggesting that early weaning may provide significant benefits through a rapid transition to artificial diets.

The effect of light intensity on weaning of golden perch fingerlings was tested, with bright light, low light level and no light treatments. Golden perch weaned best in low light (1.79 ± 0.081 lux) and bright light (73.64 ± 0.55 lux), but over 6% of those in the dark treatment (0.00965 ± 0.00275 lux) were weaned successfully. The low light treatment produced significantly better results in terms of condition (3.45 ± 0.01 compared to the bright treatment (2.9 ± 0.01) ($P<0.05$), but was not significantly different in terms of survival or growth.

Two experiments were conducted on weaned golden perch to determine effects of density and diet on growth of golden perch in tanks. Firstly, in order to test the effect of density on growth of fingerlings, and to determine if density used in the weaning trials was suitable, golden perch fingerlings were grown in tanks at densities of 1000, 2000,7500 and 10000/m^3 for 82 days. At high stocking density there was less heterogeneity in growth than at low density, but overall growth was slower. Fish in the highest density treatment weighed significantly less (5.9 ± 0.3 g) than other groups (7.1± 0.3 g to 7.9 ± 0.4 g). Secondly, due to perceptions in industry that pellet texture was an impediment to golden perch feeding, a soft pellet was prepared using gelatine as a binding and moistening agent, and tested against the three commercially available dry pelleted feeds. Growth of golden perch fed on moist pellets (2.44 ± 0.07 g to 2.6 ± 0.07 g) was significantly less than that of fish fed dry pellets (3.4 ± 0.09 g and 3.83± 0.09 g).

After the issues in weaning golden perch on to artificial foods were resolved, trials to assess growth rates of golden perch in pond culture conditions were undertaken . The initial trial was conducted at two densities (105, 000 and 31, 250 fish/ha) for 220 days, with two replicates for each treatment. There were differences between the treatments in terms of growth (low densities 96.86 ± 9.62 g and 121.92 ± 10.61 g; and high densities 83.75 ± 10.01 g and 89.02 ± 10.65 g), but only the heaviest high density treatment was significantly different to the others. The size frequency distribution of high density treatment was skewed to the left (i.e. a high proportion of small fish) and bimodal, whereas in low density treatments it was more normal. To determine the reason for the skewed distribution the density experiment was repeated with greater replication (3 replicates of each treatment) and fish were sampled regularly to determine the role of diet in growth patterns. The results showed that a large proportion (67-70%) of fish reverted back to eating natural foods and that these were generally much smaller (mean weight about 10g) than those which retained pellet eating behaviour (mean weight around 80g). For every percentage point of pellet in the gut the weight was on average increased by 0.6423%. Analysis of natural diets determined that golden perch are more selective feeders than previously thought with smaller fish selecting *Moina* as prey over copepods, and larger fish feeding on chironomids or Trichoptera but not on Ephemeroptera or Odonata.

In order to test whether exposure to formulated food had a major influence on retention of weaned golden perch on pellets, a further experiment was run to test the effect of broadcast feeding. The results indicated that broadcast feeding significantly enhanced retention of fingerlings on pellets (42.5% retention in broadcast fed treatments against 25% in point feed treatments) and overall growth rates were therefore improved. Broadcast fed fish (15.639 ± 1.07 g) were significantly larger than point fed fish (10.74 ± 0.52 g and 10.899± 1.14 g) at the end of a four month nursery period. In addition, a commercial probiotic product was concurrently trialed to determine whether probiotics had positive effects on water quality or health of fish. The results were too variable to permit meaningful analysis, due to the inherent variability of pond based production systems.

Growout of golden perch to market size after nursery phase was also conducted. To determine whether the smallest golden perch did have growth potential in a commercial setting, the entire contents of six ponds of fish were graded after nursery phase into the smallest 50% and the remainder. The different groups were then restocked into separate ponds. Ungraded controls (at the original density of 1265 fish/pond, approximately 4 fish /m^2) were maintained as a control group. Sex ratios of the respective populations suggested that there was selective mortality of the fastest growing females due to grading (70% males in graded treatments compared to 62% males in the ungraded treatments**)**. The majority of the small size class of fish did not reach market size in the six months after grading. Small fish started at 6.2 ± 0.4 g finished at mean weight of 107.6 ± 10.83 g, compared to large fish stocked at 15.7 ± 0.2 g which grew to a mean weight of 235.1 ± 20.56 g. Ungraded fish averaged 10.9 ± 1.14 g at the start of the experiment and averaged 165.7 ± 22.43 g at the end. Small fish did not appear to grow rapidly when separated from potentially dominant, larger fish, suggesting that factors other than behaviour influenced the size frequency distribution of golden perch cultured in ponds

Finally, when a mixed motile Aeromonad and hymenostome ciliate infection destroyed fish in early growout trials during this study, the aetiology and pathology of the disease was documented and an effective treatment devised. It was determined that *Tetrahymena corlissii* is a primary pathogen to naïve golden perch, and that

motile aeromonad bacteria were probably secondary invaders. An effective treatment using a systemic protozooicide was administered which halted mortalities. Previously, *Tetrahymena* had not been reported in Australia, or in food fish, as a primary pathogen of fish in well managed ponds.

In summary, the results of this study indicate that golden perch can be weaned on to artificial foods and do have potential for aquaculture, although there are still issues regarding feeds and feeding (particularly retention of artificial foods), and handling of fish (and subsequent losses due to infections), which require further research for the industry to develop rapidly. However, their potential rapid growth, high market price, and tolerance of poor water quality engender them to profitable aquaculture production systems.

Table of Contents

List of tables

List of Figures

Chapter 1
Introduction and Literature Review

1.1 Scope of Review

The scope of this review is to cover the biology of golden perch *Macquaria ambigua* (Richardson), concentrating on the aspects of its behaviour and ecology that are important in developing aquaculture techniques for growout of this species. Research has focussed on the biology of the species in the wild, and studies on feeding, physiology and reproduction in experimental situations.

1.2 Importance of golden perch

Golden perch are members of the family Percichthyidae or 'temperate bass'. The Percichthyidae contains four freshwater Australian genera (*Macquaria, Maccullochella, Bostockia* and *Guyu*). The genus *Macquaria* probably arose from a marine ancestor that became trapped in the Australian inland sea in the late Cretaceous period (about 80mybp) (Musyl and Keenan, 1992).

Golden perch are an esteemed angling fish widespread throughout central Australia, inland south eastern Australia, and coastal Queensland. They are the most important recreational and commercial native, completely freshwater fish in Australia (Pollard *et al.*, 1980). They are regarded as Australia's best freshwater table fish (Lake, 1971; Cadwallader and Backhouse, 1983) and have historically supported extensive commercial fisheries (Pollard *et al.*, 1980; Pusey *et al.*, 2004). The consumer preference for golden perch may in part be due to the absence of muddy taints common in many other freshwater fish (Marshall *et al.*, 1959). Golden perch are a popular angling species, and their decline in the wild drove research to centre on ecology, and developing and improving fingerling production techniques. Large numbers are produced and stocked into impoundments and rivers by fisheries agencies in Victoria and New South Wales, and private hatcheries in Queensland (Table 1·1).

Historically, golden perch were harvested from the Murray River and its tributaries in New South Wales, South Australia and Victoria. Reported commercial landings of golden perch from 1976-1984 ranged from 77-242 t/annum in New South Wales, 38.3-200.5 t/annum in South Australia, and 3-9t /annum in Victoria (Cadwallader, 1985). Tonnages sold between 1997/98 and 2003/4 are presented in Table 1.1.

The wild fishery supply is diminishing (Table 1·1), indicating that aquaculture product could fill the void. Historically, the majority of golden perch sold in registered markets come from the Murray River in South Australia, and the Darling River in New South Wales. The New South Wales commercial fishery ceased in 2001 (Mosig, 2001) and the only source for markets is from South Australia and, potentially, aquaculture. High quality fish consistently fetch prices over $12/kg (Ruello, 2000; Graham, 2004a). Recent market assessment has indicated that aquaculture produced golden perch is regarded equally with wild caught product and fetches a small premium ($15-19/kg) over the wild caught fish ($12-15/kg) (Graham, 2004a).

Table 1·1. Golden perch production figures in Australia. Tonnage sold is exclusively wild caught fish, except for 2003/2004 which is aquaculture product.

Year	Tonnage sold	Fingerlings (thousands)	Value ($) (thousands)	Source
1997/98	87.1	1174.8	1683.5	O'Sullivan and Roberts (1999)
1998/99	5.7	2096.0	403.3	O'Sullivan and Dobson (2000)
1999/2000	4.8	3549.8	2508.8	O'Sullivan and Dobson (2001)
2001/2002	3.5	1032.6	240.4	O'Sullivan and Savage (2004)
2003/2004	1.8	1061.4	300	O'Sullivan *et al* (2006)

Thus, there is considerable potential for development of a viable golden perch aquaculture industry, providing that obstacles of their perceived reluctance to take formulated foods (Anderson, 1986; Fallu and Mosig, 1994) can be overcome.

1.3 Biology of golden perch

1.3.1 Distribution

Golden perch were historically distributed throughout the Murray-Darling River system, the Bulloo internal drainage system, the Lake Eyre catchment, and the Fitzroy River in Central Queensland (Musyl and Keenan, 1992). Golden perch are the most northerly distributed native Percichthyid fishes, with the exception of *Guyu wujalwujalensis* (Pusey and Kennard, 2001). Their presence in central Queensland is due to river capture, specifically geological uplift redirecting inland river flow to the coast (Pusey *et al.*, 2004).

In the Murray-Darling system dams and weirs have restricted movements, reduced water temperatures and decreased flood events, thus impeding spawning and recruitment of golden perch (Lake, 1971; Battaglene and Prokop, 1987; Brumley, 1987; Rowland, 1996). As a response to the decline of golden perch in the wild, millions of fry have been stocked into public waters, primarily impoundments, since techniques for mass rearing were developed in the late 1970s (Battaglene and Prokop, 1987; O'Sullivan and Savage, 2004). Golden perch have been extensively translocated throughout their natural range, and beyond their range into south-eastern and northern Queensland, and the Northern Territory (Merrick and Schmida, 1984). A feral population has established in the Burdekin River in Northern Queensland.

1.3.2 Breeding

Spawning and breeding of golden perch has been studied extensively, as it was necessary for the development of formulated rearing techniques. Rising water temperatures are thought to stimulate gonad maturation of golden perch, and flooding is considered to be the primary natural spawning cue when the temperature is above 23ºC (Lake, 1967; 1971; Humphries *et al.*, 1999). They may spawn at slightly lower temperatures in large floods (Lake, 1971). Observed spawning events have occurred around dusk between September (Battaglene, 1991) and March (Lake, 1967). Resumption of feeding after starvation induces gonad maturation and elevated reproductive hormone levels outside the ‘normal’ breeding season, indicating that

food availability plays a critical role in reproduction of golden perch (Collins and Anderson, 1999).

Lake (1967) developed techniques for spawning in which flooding was simulated. The inference from this was that inundation of dry ground produced a factor that stimulated spawning, based on observations of recruitment after floods. More recent research indicated that flooding in addition to temperature, is essential for spawning, and that floods influence recruitment through availability of food for the larval fish (Battaglene, 1991). The validity of exclusively linking flooding to spawning and recruitment has been questioned, as in-channel habitats (as opposed to floodplain) may be used by many species for spawning and recruitment, even during low flow periods (Humphries *et al.*, 1999).

Mature oocytes are 1 mm *in vivo*, and swell to 4 mm diameter when spawned (Rowland, 1995a). The eggs are planktonic (Lake, 1971). Fecundity is very high, with a 2.5 kg female producing up to 500 000 eggs (Lake 1971) shed in a single spawning event (Cadwallader and Backhouse, 1983). The larvae hatch three days after spawning, and are small and poorly developed, similar to some marine fishes (Arumugam and Geddes, 1987; Rowland, 1995a).

1.3.3 Feeding in fingerlings and juveniles

Golden perch juveniles in farm dams are primarily insectivorous (Barlow *et al.*, 1987). One year old golden perch (188±100 g) eat mainly Corixid nymphs, with significant quantities of Notonectids, Dytiscids, Trichoptera and Plecoptera (Barlow *et al.*, 1987). Two year old golden perch (289±65 g) feed predominantly on Trichoptera nymphs, (over 60% of the diet), followed by crayfish (13%) and Coleoptera (7%) (Barlow *et al.*, 1987). Young (unspecified age) golden perch feed on zooplankton, mainly copepods and cladocerans; older fish feed mainly on large crustaceans, such as shrimps and yabbies, as well as insect larvae, molluscs and fish (Cadwallader and Backhouse, 1983).

A comprehensive study of golden perch in an impoundment in NSW (Lake Keepit) and inflow river (Namoi River) indicated that golden perch are generalised,

opportunistic, macrophagic carnivores (Battaglene, 1991). In the dam the diet was mainly fish (37%), Crustacea (33%) and Insecta (19%). Bony bream (*Nematalosa erebi* (Günther)) consumption was linked to recruitment as the size class most common at the time was most abundant in guts of golden perch. Other fish eaten included smelt (*Retropinna semoni* (Weber)), gudgeons (*Hypseleotris* sp.), carp (*Cyprinus carpio* Linnaeus) and goldfish (*Carassius auratus* Linnaeus). Crustaceans eaten were mainly *Macrobrachium australiense* and *Paratya australiensis.* Insects eaten were primarily Corixidae and Notonectidae. Feeding activity peaked in winter, declined in spring, increased in summer, and declined in autumn. There was a seasonal dietary cycle, in autumn and winter fish were 25% or more of diet, while crustaceans were consumed mainly in spring and summer. The seasonal change in diet was attributed to more sick bony bream being available in the dam in cooler weather. In the river crustaceans predominated in the diet of adult fish (87%, with yabbies making up 63%). Juvenile golden perch ate smaller crustaceans (*P. australiensis* 47·8%) and fish (*Hypseleotris* 17% and smelt 10·4%). All golden perch in all environments were active feeders at dawn and dusk.

These studies indicate that golden perch are opportunistic predators in natural environments, eating what is most available at the time. This may have implications in feed formulations in aquaculture conditions

1.3.4 Growth rates

Growth of golden perch fry (0.47 mg to 691 mg) is rapid (SGR 15%/day) being exponential for both length and weight, which in part may be attributed to the small size of golden perch at hatching (Arumugam and Geddes 1987). This growth rate is comparable to barramundi fry (Barlow *et al.*, 1993). Golden perch in Lake Keepit (central NSW) can grow to the preferred market size of 400-600 g in less than two years (Table 1·2). This, combined with the low fingerling price and high market value (when compared to barramundi or silver perch), bode favourably for development of golden perch aquaculture.

Table 1·2. Growth of golden perch in rivers and impoundments in NSW. Total length in mm unless indicated otherwise. Weight measurements where calculated are in parentheses.

Year	Murray River[1]	Darling River[3]	River[4]	Dam[1]	Lake Keepit-female[2]	Lake Keepit-male[2]	Farm Dam[5]	Lake Eppalock[6]
1	160	170	162		227.3 (198.3g)		188± 100g	
2	280	300	281	350	349.1 (740 g)	353.8 (788 g)	285±65g	300 (540 g)
3	370	380	368	390	392	372		400(1.1 kg)
4	420	430	425	440	439	426		460(1.8 kg)

[1] Cadwallader and Backhouse (1983); [2] Battaglene (1991); [3] Reynolds (1976); [4] Jones (1974); [5] Barlow *et al*. (1987); [6]. Sissins (2004). Weights in parentheses calculated using the equations of Battaglene (1991). F $W=1.155*10^{-5}*L^{3.07}$; M $W=2.496*10^{-4}*L^{2.55}$.

The length:weight relationship for golden perch is different for males and females (Battaglene, 1991). "Females were heavier at any given length than males, grew larger and showed less variability in weight at any given length" (Battaglene 1991, p 60). However, if the equations developed by Battaglene (Table 1·2) are used to calculate weight from length, the results indicate that males are heavier than females. Thus, it must be assumed that the weights calculated from the above are in fact reversed, the weights assigned to females are in fact those for males and vice versa.

1.3.5 Ecology

The natural and feral distribution of golden perch is catchments with large, seasonally inundated floodplains. They are found from clear head water streams to turbid sluggish rivers, dams and billabongs (Battaglene, 1991; Rowland, 1995a), but prefer turbid waters (Merrick and Schmida, 1984; Pusey *et al.*, 2004). Golden perch have been stocked into many impoundments, where they grow but do not establish self-sustaining populations (Rowland *et al.*, 1983). Radio tracking studies have shown that golden perch prefer water 6-16 m deep and structure (fallen trees, rocks, debris) (Anonymous, 2003).

Temperature tolerances of golden perch range from 4-37°C (Merrick, 1976), although feeding activity is reduced below 16°C and above 30°C (Sissins, 2004). Golden perch have a high tolerance of saline water (to 33‰) (Llewellyn and MacDonald, 1980), which may reflect their recent marine ancestry.

Gonad maturation is associated with resumption of feeding after starvation (Collins and Anderson, 1999). Extreme changes in availability of food can determine the capacity of golden perch to complete oogenesis (Collins, 1996). The inference from this is that flooding allows gonad maturation and spawning about one month after resumption of feeding.

The ecology of golden perch will have benefits and disadvantages in aquaculture systems. The ability to grow rapidly at times of abundance of food, and their apparently opportunistic feeding habits, suggest that when supplied a nutritionally adequate diet at appropriate levels they should grow rapidly. Their ability to withstand long periods of food deprivation, but recoup lost weights and energy reserves quickly once feeding resumes, suggests that feeding strategies to reduce overall food consumption but increase food assimilation could be developed. The ability of golden perch to live in turbid water also augers well for aquaculture in inland Australia, where turbid water is common.

A possible drawback of the linkage between food availability and breeding is that golden perch may divert energy to gonad maturation earlier than would be the case in the natural flood and food driven environment. Although this may not lead to energy expenditure in spawning, it does divert energy away from growth. This would not be a desirable consequence in an aquaculture situation with continuous food availability.

1.4 Taxonomy and Genetics of Percichthyidae in Australia

The genetic structure of golden perch throughout their distribution (Section 1.3.1) has been well studied (Musyl and Keenan, 1992; Keenan *et al.*, 1995; Tikel *et al.*, 2002). Protein electrophoresis suggests that there are three distinct genetic stocks of golden perch in Australia (Musyl and Keenan, 1992). The Murray-Darling and Fitzroy River populations are closely related (the Fitzroy race, *Macquaria ambigua oriens*; and

Murray-Darling strain *M. ambigua ambigua*) but the Lake Eyre population was classed as a distinct species (Musyl and Keenan, 1992). The Bulloo population appears to be intermediate between Murray-Darling and Lake Eyre populations. The information presented and lack of drawings or referral to meristic characters preclude these names from being used as taxonomic terms, and for the purpose of this thesis the terms golden perch and *Macquaria ambigua* refer to the Murray-Darling strain unless otherwise indicated.

Conversely, mDNA studies indicate that the Fitzroy population is distinct at the species level, and the Lake Eyre and Murray-Darling populations are subspecies (Tikel *et al.*, 2002). The feral Burdekin River population of golden perch has Murray-Darling ancestry (Tikel *et al.*, 2002). However, mitochondrial DNA illustrates maternal ancestry, and cannot identify the occurrence of hybridisation. Tikel's study was not taxonomic in nature, but an investigation of genetic diversity of hatchery stocks, and did not formally describe or name the species or subspecies of golden perch identified.

The contradiction in results between the mDNA and electrophoretic research indicates the need of a review of the taxonomy of golden perch to ascertain the true relationships within the species, or whether it is a species complex, as this will have important applications in hybridisation or outbreeding for aquaculture use.

There are seven genetically distinct populations of golden perch (determined using protein electrophoresis) within the Murray-Darling basin, including three impounded populations that may be artefacts of restocking or restricted migration (Keenan *et al.*, 1995). It is also of significance in aquaculture, as specific clines may be amenable to culture.

Therefore, there are distinct populations of golden perch within river basins, and significant differences between river basins. This suggests that characteristics favourable for aquaculture could be identified and used, and that hybridisation or selective breeding could be employed to obtain rapid improvements in aquaculture production. The colour and meristic differences between populations of golden perch have not been adequately documented. Differences in body shape or colour are critical

in marketing. Currently the market expects and accepts Murray-Darling strain golden perch as the benchmark (Ruello, 2000). If other varieties are to be used, a full understanding of demands and expectations of the market or alternative marketing strategies will have to be considered.

1.5 Golden perch in experimental and aquaculture situations

1.5.1 Induced breeding and fingerling production

Due to the reduction in numbers of golden perch (among other inland species) efforts to propagate them were developed by NSW Fisheries and have been adopted by both private and state sector fish hatcheries in Queensland, Victoria and New South Wales. Initially, production was achieved by simulating natural flooding and temperature increases (Lake, 1967). Subsequently, techniques for hormone-induced spawning were developed (Bowerman, 1975) and are now the method of choice (Rowland, 1983a; 1983b). The method involves application of hormone (HCG, human chorionic gonadotropin; or CPH, carp pituitary hormone) to gravid females, which are then placed in tanks with males. HCG and Ovaprim are commonly used in hatcheries because of ease of use and ready availability. Spawning usually occurs within 36 hours and the planktonic eggs are moved to hatching tanks. Fry are then transferred to larval rearing ponds, prepared to have a plankton bloom when fish fry are introduced (Rowland, 1995a; Rowland, 1996). This has resulted in reliable production of millions of golden perch fingerlings on an annual basis (Table 1·1). The availability of appropriate sized food at first feeding is critical for larval survival in production ponds (Arumugam and Geddes, 1987). Golden perch are, numerically, the dominant freshwater fish species produced in Australian fish hatcheries, with over 3.5 million fingerlings produced in 1999/2000, and 1.06 million produced in 2003/2004 (O'Sullivan and Roberts, 2001; O'Sullivan *et al.*, 2006).

1.5.2 Feeding of fingerlings in aquaculture ponds

Golden perch larvae initially feed on slow moving rotifers and cladocerans, changing to faster moving and larger prey as their size and mouth gape increases (Arumugam, 1986a; Arumugam and Geddes, 1987; 1988). The ability to swallow prey determines

what golden perch larvae and fry eat, as they are unable to swallow prey larger than the gape, but do not select food items based on size as larvae (Arumugam and Geddes, 1987). As the fry grow, larger *Daphnia* become the dominant food item (Arumugam and Geddes, 1988). Survival of golden perch larvae stocked into plankton ponds was significantly better if they were fed in the laboratory (on plankton or *Artemia*) for two to three days before release into ponds (Arumugam, 1986b). In commercial operations, first-feeding larvae are introduced into ponds rich in plankton and grown to 30-50 mm total length (TL) in 4-8 weeks (Rowland, 1983b; 1996).

1.5.3 Nutrition and feeding

An early study aimed at elucidating the biology of golden perch for breeding encountered substantial problems in finding an easily available food that was palatable (Stevenson and Grant, 1957). Stevenson and Grant eventually settled on using mud whelks, but actually found that crustaceans were the preferred diet. They also substantially reduced supplementary feeding by promoting natural production in the pond.

Previous studies of golden perch nutrition have been hampered by the difficulty in weaning golden perch on to formulated diets. Attempts to determine lysine requirements (Logan, 1986) and optimal protein level (Sloan, 1986) were unsuccessful as they were unable to wean the fish onto either fish meal or experimental diets.

Nichols (1994) studied the effect of enriched earthworms (*Eisenia foetida supermorpha*) as a feed for golden perch. Poly Unsaturated Fatty Acid (PUFA) (ω3 acetyl fatty acids)- enriched earthworms resulted in enhanced growth rates. *E. foetida* were efficiently digested, and presented a potentially viable food resource for golden perch farming (Nichols, 1994). This study was undertaken because golden perch had not been successfully weaned onto a formulated pellet.

Mc Fayden (2001) investigated protein requirements of golden perch using weaned Murray-Darling fish (supplied by the Freshwater Fisheries and Aquaculture Centre,

Walkamin), and diets varying from 30% protein to 55% protein, in 5% increments. Digestibility (indicated by ytterbium) indicated that protein uptake plateaued at a level of 40%, suggesting that diets with higher levels of protein are not necessary.

1.5.4 Diseases

The diseases of golden perch have been relatively well documented due to breeding programs and the importance of the species in recreational fishery enhancement. Table 1·3 lists diseases recorded in golden perch by NSW and Victorian Fisheries (Beumer *et al.*, 1982; Rowland and Ingram, 1991), and the Queensland Department of Primary Industries and Fisheries.

Table 1·3. Diseases and parasites of golden perch.

Disease agent group	Species	Effect	Stage of golden perch affected
Protozoans	*Ichthyophthirius multifiliis*	Skin lesions, rapid death	Fry most severe
Protozoans	*Chilodonella hexasticha*	Skin lesions, rapid death	Fry and adults
Protozoans	*Trichodina* sp.	Gill damage	Especially larvae, fry
Protozoans	*Ichthyobodo necator*	Gill damage	Tank reared fish
Protozoans	*Tetrahymena corlissii*	Skin lesions	Juveniles and adults
Protozoans	*Goussia langdoni* or *Eimeria ashburni*.	Coccidiosis (gut lesions)	Fry
Copepods	*Lernaea cyprinacea*	Skin damage, emaciation	All stages
Fungi	*Saprolegnia* and *Achlya*	Skin damage	Damaged fish
Bacteria	*Aeromonas hydrophila*	Bacterial haemorrhagic septicaemia	All fish
Bacteria	*Aeromonas sobria*	skin ulceration, cutaneous petechial haemorrhage, septicaemia	All fish
Bacteria	*Flavobacterium columnare*	Saddleback skin lesions	Juvenile fish
Nematodes	*Spirocamallanus murrayensis*	Parasitic roundworms	All fish
Nematodes	*Eustrongylides* sp.	Parasitic roundworms	All fish

The diseases listed in Table 1·3 have primarily been recorded from fish held for breeding or in hatcheries. Fry are particularly vulnerable to parasitic infections due to the unnaturally high densities in rearing facilities. Of particular concern are cryptic or chronic diseases which are hard to detect until the infection has progressed significantly. Bacterial diseases are also of concern as many act swiftly if fish are stressed, but require antibiotic sensitivity testing (taking 24-48 hours) for reliable treatment. Bacteria are ubiquitous and can cause severe problems after fish handling.

1.5.5 Physiology

Golden perch adapt well to aquarium conditions, acclimate quickly to conditions of chronic stress, and recover quickly from stressors such as anaesthesia and exertion, (Carragher and Rees, 1994) which are common during handling in aquaculture.

Two studies on digestion in golden perch are of significance to aquaculture of the species. Anderson and Braley (1993) investigated residence times of food and appearance of amino acids in the blood of adult (326-875 g) golden perch, and reported a residence time of 96 hours. This was determined as the time taken for a polystyrene ball placed in a goldfish meal to pass through the gut of fish fasted after feeding (Anderson and Braley, 1993). However residence times in barramundi (*Lates calcarifer*) fry are over four times longer in starved fish than fed fish (Barlow *et al.*, 1993). Golden perch have slow digestive processes, as some amino acids take 18-36 hours to appear in the blood, as opposed to 3-4 hours for some other species (Anderson and Braley, 1993). However, some amino acids appeared within three hours of feeding, suggesting that non-essential amino acids (used for energy) were absorbed before essential amino acids (Anderson and Braley, 1993).

Golden perch undergo a staged catabolic response to starvation but regenerate quickly after feeding recommences. Starved golden perch mobilise lipid stores in the liver for the first 30 days of starvation, subsequently the perivisceral fat bodies are used, followed by muscle (Collins and Anderson, 1995). After resumption of feeding, the liver reserves, followed by perivisceral fat reserves, are restored. The impact of short term starvation on weight and condition of fish is important, due to the potential effect

of weight loss during purging (an essential process for silver perch before market). The utilisation of rapidly renewable energy reserves during the first thirty days of starvation is of interest in aquaculture, as it may permit feeding strategies to minimise labour costs, while not compromising growth rates. Due to higher pay scales on weekends, avoiding feeding at these times without significant loss in growth can be economically beneficial. Such strategies have been developed for barramundi farming in northern Australia (Williams and Barlow, 1993).

1.6 Weaning of fish in aquaculture

Weaning of essentially planktivorous fish fry or juveniles on to formulated diets is one of the earliest hurdles to overcome in aquaculture development, particularly in marine fish (Devresse *et al.*, 1991; De Silva *et al.*, 2001). Live foods are used in initial feeding due to the poorly developed digestive tract, which limits digestion of complex substances (Kolkovski, 2001), or to behavioural cues which initiate feeding behaviour, such as movement of prey. Use of formulated diets for fish larvae and juveniles is preferred to dependence on live foods (Gennari *et al.*, 1994), as formulated diets can be superior to live foods (Lee and Litvak, 1996). Co-feeding live and formulated foods may assist in digestion of the formulated food (Walford and Lam, 1991). Digestion in carnivorous larvae may rely on autolysis of prey species (Jones *et al.*, 1991) and may be necessary to prime the gut with flora essential for digestion of other foods. Formulated foods, which contain binders, coatings and other complex ingredients (Partridge and Southgate, 1999), are generally poorly assimilated by the undeveloped digestive systems of many larval fish (Walford *et al.*, 1991; Baskerville-Bridges and Kling, 2000). Co-feeding during a weaning process may assist significantly in digestion and assimilation of formulated foods due to autolysis of live food (Walford *et al.*, 1991).

Studies on weaning fish have focused on weaning of larvae from live foods onto formulated diets for efficiency and improved nutrition. In temperate developed countries, intensive methods (where temperature, water quality, nutrition and environmental variables are controlled) are used to maximize production (Dabrowski *et al.*, 1984; Zitzow and Millard, 1988; Person-Le Ruyet, 1990). In tropical and subtropical freshwater environments, where land for ponds is not a limiting factor,

production of fish fry in zooplankton ponds is a viable alternative to intensive production (Rowland, 1983a; Rowland, 1995a; Rowland, 1996).

The transition period from live foods to a formulated diet is often critical in larval survival (Lee and Litvak, 1996; Aneshansley and Timmons, 2001), and weaning of most marine species involves a transition period between live and formulated feeds. In some species gradual weaning gives better survival and growth than abrupt weaning (Juario *et al.*, 1991; Cañavate and Fernandez-Diaz, 1999). Indeed, a co-feeding process involving a gradual transition from natural to formulated diet is necessary to wean a variety of fish species (Anderson, 1974; Bondari, 1983; Zakes, 1997; Jenkins and Smith, 1999; Ljunggren *et al.*, 2003; Alves *et al.*, 2006). For example, glass eels and elvers are gradually weaned using fish roe (De Silva *et al.*, 2001). No weaning process is used in pond production of some species, for example silver perch (Rowland, 1994) with the fish making the transition from natural to formulated food as natural food is depleted. To date, most marine finfish produced by commercial operations require a proportion of live or fresh food in the early feeding stages.

There is little published information on the success of weaning freshwater fish fingerlings on to formulated diets. Elvers, *Anguilla australis*, may be analogous to fish fingerlings, and require a weaning period before they will ingest formulated diets (De Silva *et al.*, 2001).

Golden perch fingerlings are reared commercially in Australia using methods developed in New South Wales (Rowland, 1995a; Rowland, 1996), viz, first feeding larvae are reared in plankton ponds until they are of a size considered best for stocking into water bodies, usually 50 mm total length (TL). To date, there has been no attempt to improve on a system that successfully supplies the existing market for native fish fingerlings. Only one commercial system has developed techniques for intensive aquaculture of golden perch, including weaning (Mosig, 2001).

1.7 Size heterogeneity and grading of fish in aquaculture

Heterogeneity of sizes of fish in aquaculture is a major management issue, as most species reared in aquaculture exhibit considerable growth variation (Martins *et al.*, 2005). Silver perch are considered an excellent species for aquaculture due to their relatively even growth, but this can be compromised by poor feeding practices (Rowland and Barlow, 1991; Rowland and Walker, 1995; Rowland *et al.*, 2001). Eurasian perch are cultured from fingerlings supplied by hatcheries (Mélard *et al.*, 1996b), which may be analogous to potential production models for Australian species for which mass, cheap supply of fingerlings is available. Growth heterogeneity constitutes a major constraint in Eurasian perch (*Perca fluviatilis*) culture (Mélard *et al.*, 1996a; Mélard *et al.*, 1996b; Kestemont *et al.*, 2003). The reasons for size heterogeneity can include development of hierarchies, or density dependent inhibition of territorial and agonistic tendencies that potentially limit access to food (Mélard *et al.*, 1996b). Another warm water species, African catfish (*Clarias gariepinus*) also exhibits great size heterogeneity necessitating grading, although the reasons for growth heterogeneity appear to be primarily intrinsic (not related to intraspecific interactions) but may be related to differential feeding behaviour of larger fish (Martins *et al.*, 2005; Martins *et al.*, 2006; Martins *et al.*, in press). The effect of group composition on feeding behaviour has been little studied in cultured fish species (Martins *et al.*, 2006). Separating smaller fish from larger fish of the same population should provide an indication of whether dominant larger fish are inhibiting growth or whether genetic or other factors are more important (Wohlfarth and Moav, 1994; Endemann *et al.*, 1997; Martins *et al.*, 2006). Certainly genetic factors are of great importance, as illustrated in European sea bass (*Dicentrarchus labrax*) and channel catfish (*Ictalurus punctatus*), parentage has major impacts on growth, sex ratios and heterogeneity(Malison *et al.*, 1988; Goudie *et al.*, 1993). Intrinsic differences in feeding behaviour of smaller and larger fish may also affect growth rates in some species (Martins *et al.*, 2006).

Grading in cultured fish is a standard management practice particularly important in cannibalistic species. It is also important in aquaculture for management reasons (homogeneity of fish sizes improves feeding efficiency, harvesting etc). However, in many warm water species it is apparent that grading does not affect growth heterogeneity, and certainly the stress associated with grading can have long lasting

detrimental effects on growth and survival in Eurasian perch, African catfish, and European sea bass (Mélard *et al.*, 1996b; Kestemont *et al.*, 2003; Martins *et al.*, 2006).

1.8 Aims of this Study

Golden perch, being a high value fish product and having a sustained demand, is an ideal candidate for aquaculture. If growth rates attained in the wild can be achieved under aquaculture conditions, then aquaculture of the species will be viable (providing food costs are similar to those for barramundi). The primary obstacle to aquaculture of golden perch has been weaning of fingerlings onto formulated diets, and subsequent transition into pond conditions. These two issues are critical for future development of aquaculture of this species, and are the major themes of this thesis.

Five areas of research are developed. These are:

- developing an efficient, reliable weaning technique for golden perch;
- refining techniques for weaning golden perch by determining environmental and other conditions for weaning (e.g. size of fingerlings, density, light intensity, duration of co-feeding, moist diets);
- investigating nursery phase production in pond systems;
- investigating the effect of grading on performance in pond aquaculture in growout to market size; and
- an opportunistic investigation of an epizootic which decimated golden perch in aquaculture conditions.

Development of techniques for weaning incorporated devising a successful weaning method (Chapter 2), then testing the effect of transition period, size at weaning and light intensity of weaning success (Chapter 3). As a follow-on from these experiments, the effect of density and formulated diet texture (moist vs dry diets) on weaned fingerlings in experimental tank systems was trialed (Chapter 4). Pond nursery stage culture techniques were investigated, focusing on effect of density initially. When issues regarding uneven growth rates were encountered, feeding behaviour in ponds and revised feeding techniques were examined (Chapter 5). To determine the length of time required to grow golden to market size, the structure of pond grown populations, and the effect of grading on growth rates of fish, was

examined to determine techniques for pond based production of golden perch (Chapter 6). The effect of grading was also examined to elucidate the role of behaviour and food in the Poisson growth distribution.

During initial growout trials, a systemic bacterial and protozoan infection of golden perch was encountered. This had a devastating effect on the fish under culture and was investigated to develop control methods for this disease (Chapter 7). Disease in aquaculture is largely a result of stress and high densities in culture conditions, and may be exacerbated when translocation (of pathogens or fish) exposes naïve fish to novel strains or types of pathogens.

The results of the experiments including the development of a weaning protocol, the effects of density and implications for grow out, and golden perch aquaculture techniques, are drawn together in the general conclusion (Chapter 8).

The results generated during this study will overcome one of the major bottlenecks in development of commercial golden perch culture and will provide baseline information which will facilitate commercial development of this lucrative freshwater fish in Australian aquaculture.

Chapter 2
Development of a weaning protocol for golden perch fingerlings

2.1 Introduction

2.1.1 Development of weaning protocols for golden perch fingerlings

To date, an impediment to golden perch aquaculture has been the inability to successfully wean fingerlings on to formulated diets (Anderson, 1986; Mosig, 2001). Although they have been maintained in tanks on natural foods (Nichols, 1994; Collins, 1996) golden perch must be trained to accept formulated diets for intensive commercial aquaculture to be successful. Training fish fingerlings to accept formulated diets by gradual replacement of the natural food with formulated diets can increase success of weaning significantly (Kubitza and Lovshin, 1997). Co-feeding (combining natural and formulated foods) improves weaning of many fish larvae and juveniles onto formulated diets (Stickney, 1986; Rosenlund *et al.*, 1997; Anguas-Vélez *et al.*, 2000).

In the tropics zooplankton is readily and easily cultured in quantity at any time of the year. Production of native freshwater fish fingerlings in Australia is largely dependent upon use of fertilised plankton ponds stocked with larval fish which feed on zooplankton. In general, fish reach a size suitable for stocking for recreational fishery enhancement (about 50 mm) in 6-8 weeks (Rowland, 1996). Appropriate-sized zooplankton for particular fish fingerlings can be selected as most ponds produce a variety of species (Arumugam, 1986b). Frozen zooplankton has potential as an alternative to *Artemia* as it is readily available, cheap, can be stored ready for immediate use when required (Graham, 2004b), and it is a familiar food to fish reared in plankton ponds.

Golden perch fingerlings are produced in large quantities (over 3.5 million in 1998/99) (O'Sullivan and Dobson, 2001) by commercial hatcheries, so there is no real benefit to wean larvae. However, weaning is critical for fingerlings. Golden perch fingerlings have been trained to accept fresh natural foods such as worms (Nichols, 1994), and larger fish will take meat such as beef heart (Collins, 1996). Golden perch have also been successfully fed molluscs in ponds (Stevenson and Grant, 1957). It should therefore be possible to wean golden perch fingerlings on to formulated diets using fresh food as a co-feeding diet.

Weaning using foods available from seafood retailers could be a viable way of weaning golden perch fingerlings without relying on zooplankton. The advantages of using this source of weaning diet are ease and regularity of supply and storage, and processing requiring unspecialised equipment. A wide range of potential weaning alternatives is available (mollusc, fish, fish roe, crustacean), and comparison of these against the weaning diet composed of zooplankton may assist in expansion of the industry to tank based culture.

A series of experiments to determine the ability of golden perch to wean on to formulated diets was designed to determine techniques and co-feeding diets for weaning. Frozen ice blocks, made up of the co-feeding diet (eg *Artemia*, zooplankton) were made and mixed with formulated diets in increasing increments for weaning in all experiments. The aim of Experiment 2.1 was to determine if golden perch could be successfully weaned onto a formulated diet, using *Artemia* as a co-feeding diet and two commercially available fish fry foods. The aim of Experiment 2.2 was to determine whether frozen freshwater zooplankton would be an acceptable alternative to newly hatched *Artemia* nauplii as a weaning transition food for golden perch. Experiment 2.3 testing alternatives to zooplankton, was trialed in two stages. In the first stage the weaning blocks did not break up while thawing. The second stage was a repeat of the first, but incorporated crushed ice into the blocks, which allowed them to break up gradually.

2.2 Materials and Methods

2.2.1 Experimental facilities

2.2.1.1 Experiment 2.1. Weaning golden perch onto formulated diets using abrupt weaning, and co-feeding with *Artemia*.

Fingerling golden perch (four weeks old 25.45 ± 0.19 mm and 0.23 ± 0.00014 g weight, n=50) were obtained from a commercial hatchery in Queensland. They were air-freighted to the Freshwater Fisheries and Aquaculture Centre (FFAC), Walkamin, north Queensland, and were treated with 10 g/L salt and 1 mg/L methylene blue for one hour. After five days quarantine, during which they were fed on live *Artemia* nauplii, fingerlings were graded and stocked into 75 L rectangular glass aquaria at a density of 50 per aquarium. Glass aquaria were equipped with aeration and flow-through water at 22°C, with a constant flow of 15 L per hour. Lighting was indirect ambient light from widows above the tanks on both sides of the room.

The golden perch were then acclimated for 10 days and fed with frozen *Artemia* nauplii three times per day. Freshly hatched *Artemia* nauplii, concentrated to a slurry, were rinsed in fresh water, then frozen in 5 mL blocks. At feeding, a frozen block was placed into a steel 1 mm mesh bag suspended from a polystyrene float at one end of each aquarium. Thawed *Artemia* sank through the mesh to the bottom of the aquarium. Feeding was to excess throughout the experiment, as indicated by uneaten food on the tank bottom 2-3 hours after feeding.

2.2.1.2 Experiments 2.2 and 2.3. Weaning golden perch using *Artemia*, zooplankton, and commercially available seafood products.

Golden perch fingerlings supplied from a commercial hatchery were air freighted to the FFAC, Walkamin. On arrival, they were subjected to a prophylactic salt (10 g/L) and formalin (70 mg/L) bath for treatment of external parasites for one hour. They were fed live zooplankton after the treatment. Round, dark green plastic tanks with a centre drain and level controlled using an external standpipe were used in this experiment. Volume of water was 100 L, supplied with flow through bore water at 93 L per hour. The base area of each tank was 0.25m^2. Supplementary aeration was supplied with a single airstone.

Oxygen was always > 6 mg/L, and pH was between 6.8-7.1 for the duration of each experiment. Tanks were cleaned daily, a minimum of one hour after morning feeding, by scrubbing with a nylon scourer, and the standpipe dropped to drain about 60 L of water off from the central, bottom outlet.

Zooplankton was collected from a plankton pond at FFAC, washed, and sieved between 250 μm and 1.18 mm size aperture, and frozen immediately in 5 mL blocks. Zooplankton consisted of *Moina micrura* (20-70% by count), juvenile *Daphnia carinata* (10-30%), and cyclopoid and calanoid copepods (20-70%). Treatment foods were presented in plastic mesh bag (3 mm) containing a 5 mL block of frozen zooplankton or *Artemia* (processed as above) was suspended near the water surface, producing a stream of food falling to the bottom as it melted. Each 5 mL block melted in approximately 7 minutes. The fish were fed three times a day, at approximately 08:00, 12:00 and 16:00h. Water was turned off for one hour during and after feeding, to avoid food being washed out. The bag was removed from the tank one hour after each feeding, cleaned, and sun dried.

Ambient light from windows was dispersed evenly across the experimental area by use of black plastic curtains. Light intensity was measured using a Hobo light meter, which read a factor of six lower than standard photographic meters (Section 3.2.3). Average light intensity was 1.85 lux between 08:00 and 18:00h. Lights were turned on for cleaning, and light intensity during this time was 8.5 lux. Behaviour of the fish was observed during feeding and cleaning, mortalities were collected and recorded, and the apparent reason for death noted. Animals that had starved (did not wean) were noted as such. A sample of fifty fish was measured as the start point due to sensitivity of fish to handling. At the end of the experiment, all surviving fish were measured in mm and weighed in grams accurate to two decimal points. Fish that were robust in appearance were assessed as ‘feeders’, and those that were emaciated or thin as ‘non-feeders’. Fish were anaesthetised with Aqui-S, (isoeuganol anaesthetic, Aqui-S Ltd., Lower Hutt, New Zealand), standard length measured, then blotted on a damp sponge before being weighed. All measurements are mean standard length and weight ± standard error.

2.2.2 Diets and weaning régime

2.2.2.1 Experiment 2.1. Weaning golden perch onto formulated diets using abrupt weaning, and co-feeding *Artemia.*

Once acclimated, one of five treatments with three replicates per treatment were fed:

- frozen *Artemia*;
- a commercial diet Lansy ω3 (INVE, Dendermonde, Belgium[1]) co-fed with *Artemia*;
- Lansy ω3 diet without co-feeding;
- commercial salmon crumble (Gibson's Ltd, Rosny Park, Tasmania[1]) co-fed with *Artemia*; or
- salmon crumble without co-feeding.

The co-feeding treatments were made by mixing *Artemia* slurry (rinsed in fresh water) with the crumble, in 10% increments per day by volume (i.e. first day 90% *Artemia*, 10% crumble, last day 10% *Artemia* and 90% crumble)(Table 2·1). The blocks were frozen immediately after mixing and kept frozen until placed into aquaria for feeding. Proximate composition of the dry diets is presented in Table 2·2. Diets were analysed at the Animal Research Institute, Yeerongpilly, Queensland, a NATA accredited laboratory. Feeding was slightly to excess (as determined by uneaten food present after each feeding time) for the duration of the trial.

The treatments without co-feeding were fed the dry diet, starting the morning of first day of weaning. The co-feeding transition period lasted ten days. Both dry diets used were 0.6 mm size crumbles. All diets were provided three times a day, at 08:00, 12:00 and 16:00. Tanks were scrubbed and siphoned clean between 09:00 and 10:00 each day. After the co-feeding period, all treatments (except the *Artemia* control) were fed their respective dry diet for the remainder of the experiment. Mortalities were recorded daily, and where possible examined post-mortem for disease. All aquaria were emptied and each fish measured after 75 days, at the end of the experiment.

[1] Use of trade or manufacturer's name does not imply endorsement

Table 2·1. Feeding schedule for golden perch weaning Experiment 2.1.

Day	Food provided	
	Artemia %	Formulated food %
1-10 (Acclimation)	100	0
11	90	10
12	80	20
13	70	30
14	60	40
15	50	50
16	40	60
17	30	70
18	20	80
19	10	90
20-75	0	100

Table 2·2. Analysis of diets used in weaning trials.

Composition	Lansy ω3	Salmon crumble
Protein (%)	60	55
Lipid (%)	14.5	15.4
Ash (%)	11.5	11.8
Fibre (%)	7	4.1
Carbohydrate (%)(calculated)	7	13.7
Moisture (%)	7	8.6
Vitamin A (IU/kg)	30,000	28,600
Vitamin E (mg/kg)	400	236

Survival data were arcsine transformed then analysed using ANOVA and LSD of means on a Genstat 6.1 program (Lawes Agricultural Trust, Rothamsted Experimental Station, 2002). Weight and length at the end of the experiment were compared using least significant difference (LSD) tests. Means were pooled across all replicates after testing for no significant difference within treatments between replicates. Differences

between treatments were considered to be significant when $P<0.05$, unless otherwise stated. Mean weights and lengths for each treatment were also compared using ANOVA and LSD of means on Genstat 6.1.

2.2.2.2 Experiment 2.2. Comparison of *Artemia*, frozen zooplankton and formulated foods for weaning fingerlings of golden perch.

Fish were graded using a bar grader and then 75 fish of mean total length 23.9 ± 0.54 mm, mean weight 0.16 ± 0.0097 g (mean ± SE, n = 40), were stocked in each of 16 tanks. Water temperature was 24 to 27.8°C (mean 25.7°C). *Artemia* were hatched and harvested as newly hatched nauplii, rinsed in fresh water, and frozen in 5mL blocks. This amount was slightly to excess, as determined by uneaten food left one hour after feeding.

After an eight-day acclimation period when fish were fed frozen *Artemia* or zooplankton, weaning was commenced. The experiment ran for 56 days after the acclimation period.

Weaning diets were made by mixing fresh zooplankton or *Artemia* slurry with 0.6 mm salmon crumble (Gibson's Ltd, Rosny Park, Tasmania), then freezing it in blocks. Proximate analysis of the crumble was 55% protein, 15.4% fat, 4.1% fibre, 11.8% ash and 13.7% carbohydrate. During the 10 day transition, the slurry was gradually replaced with crumble over 9 days, in 10% increments by volume (as in Table 2·1). When the feeding régime was 100% crumble, the empty bag was placed in the tank as normal and 5mL of crumble sprinkled on the water surface above it. Three days after transition, the bag was no longer used and food was sprinkled on the surface at feeding times.

Treatments were:

- zooplankton fed control;
- zooplankton fed then gradually weaned on to salmon crumble;
- *Artemia* fed then gradually weaned on to salmon crumble; and
- zooplankton fed then abruptly transferred to salmon crumble. The abrupt transfer to salmon crumble was conducted to confirm the need for a transition period of co-feeding natural and formulated food.

There were four replicate tanks of each treatment. During the acclimation period, mortalities were replaced from an identical tank to experimental tanks (same volume, number and size of fingerlings). After that, mortalities were recorded daily. The experiment was terminated after 56 days, and all fish measured and weighed. Fish were divided into starved (those with a sunken abdomen) and feeding (those with a distended abdomen). When weights of these two subjective conditions were compared, starved fish were <0.8 g and feeding fish >1 g.

Data were analysed using ANOVA. Residuals were checked for departures from the assumptions, and any outliers were removed if discovered. Percentage data (mortalities, starved and feeders) were arcsine transformed before analysis. All ANOVAs were then checked for accuracy using GLM (Generalised Linear Model) analysis.

2.2.2.3 Experiment 2.3. Weaning golden perch fingerlings using zooplankton and commercially available seafood products.

Eighteen 100 L tanks were each stocked with one hundred fingerlings. Water temperature in the system ranged from 23.1 - 26.2°C; mean 25.2 °C, based on hourly logging records. Three replicates of each treatment were randomly assigned tanks, in a completely randomised design. Food (except zooplankton and roe) was prepared as follows:

- shells, head and fins (where applicable) were removed and discarded;
- the whole animal was then placed in a food processor and chopped to a slurry;
- this was then washed through a 1.2 mm Endicott sieve;
- the material that passed through was retained using a 250 μm sieve, washed, and then frozen as an ice block.

Zooplankton was collected in an airlift collector from a pond, sieved through a 1.2 mm Endicott sieve, retained in a 250 μm sieve, and frozen as slurry. Flowery cod fish (*Epinephelus fuscoguttata*) roe was prepared by scraping out connective tissue and membranes, and freezing the ova in blocks. The size of the ova was about 800 μm diameter. All ice blocks were 5 mL in volume, which was found to be sufficient for the number of fish at that size.

Fingerlings were fed frozen zooplankton or alternative (seafood) for the acclimation period of seven days, then fed a mix of zooplankton slurry and 1mm salmon starter crumble mixed in 10% increments (by volume) daily, as per Experiment 2.2. The length and weight data were analysed using ANOVA in Genstat 6.1. Pair wise comparisons were made using least significant differences. Treatments with no survivors, or not enough survivors in enough replicates, were excluded from the analysis. Survival data were analysed using logistic regression (using a generalised linear model GLM with binomial error and logit link). Predicted survival proportions were calculated and pair wise comparisons made between the treatments.

Behaviour of fish during and after feeding was observed to determine if habituation had occurred, and if fingerlings were feeding on food while sinking and after it had settled on the bottom. Observation of fingerlings during feeding was also important to determine if dominance or aggressive interactions were apparent.

Experiment 2.3-Trial One

The day after arrival, the fingerlings were graded through an adjustable bar grader, to a mean size of 31.96 ± 2.15 mm SL and 0.70 ± 0.14g weight (n=50). The fish were offered the different types of fresh foods two hours after introduction into the 100L tanks, and during the acclimation period of one week. The control was the zooplankton weaning treatment (Section 2.2.1.2). Fingerlings were fed frozen zooplankton or alternative for the acclimation period, then fed a mix of zooplankton slurry/alternative and 1mm salmon starter crumble mixed in 10% increments (by volume) daily until 100% crumble was being fed. Alternatives to zooplankton trialed were pilchard (*Sardinops neopilchardus*), scallop (*Amusium balloti*), prawn (*Penaeus plebejus* and *Metapenaeus macleayi*), and fish roe (gold spot cod, *Epinephelus coioides*). An additional treatment, where fingerlings were weaned off zooplankton on to pilchard over a six day period, was also used. When the weaning transition period was complete, the fish were fed on crumble for a further 44 days, to ensure that the fish that did not wean would be easily distinguished from the feeding fish.

Experiment 2.3-Trial Two

Methods used were the same as in trial one, except for the following differences. The day after arrival fish were graded to an average standard length of 18.14 mm ±0.227 and weight of 0.14 g ± 0.0045 (n=50). Alternative seafood blocks were prepared as above, except that 5 mL of slurry was mixed with crushed ice into a 20 mL ice block. Fingerlings were fed frozen zooplankton after stocking in tanks for ten days to habituate to feeding times and activity. Then the fish were abruptly changed to the appropriate test co-feeding food for ten days before weaning. Trial one was repeated with blocks of minced seafood mixed with crushed ice, which allowed the blocks to fall apart readily as they thawed. Due to seasonal variations in availability of seafood, pilchard (*Sardinops neopilchardus*), mussel (*Mytilus* sp.), prawn (*Penaeus plebejus* and *Metapenaeus macleayi*), and squid (*Loligo opalescens*) were used in this trial. The fish were fed on crumble for a period of 25 days after weaning was complete, as daily mortalities of non-weaned fish had declined 7 days prior.

The experiment was approved by the Far North Queensland Animal Ethics Committee. Approval number FNQ- 06-00.

2.3 Results

2.3.1 Experiment 2.1. Weaning golden perch onto formulated diets using abrupt weaning, and co-feeding Artemia

Survival over the 75 days was significantly higher for fish fed the control diet of frozen *Artemia* (86.4%) compared with those on the co-feeding treatments (71.6 and 77.6%) (Table 2·3). The two abrupt weaning treatments both had significantly lower survival ($P<0.05$) (38 and 51.2%) than the co-feeding treatments and the control. There was also a significant difference between co-feeding treatment 2 (Lansy and *Artemia*)(77.6%) and the control (86.4%) ($P<0.05$).

Table 2·3. Mean (±SE) weight and length of golden perch in different weaning treatments. The same superscript indicates no significant difference between means in the same column (LSD P>0.05). Data are means of treatments.

Treatment	Mean weight (g)	Length (mm)	Survival (%)	Feeders (%)
1 Control	1.29 ± 0.14 [c]	46.19 ± 1.53 [d]	86.4 ± 5.15 [c]	77.0 ± 1.85 [a]
2 Lansyω3 + *Artemia*	0.63 ± 0.13[b]	38.06 ± 2.01 [c]	71.6 ± 5.04 [b]	57.9 ± 5.24 [c]
3 Lansyω3	0.42 ± 0.079 [a]	33.74 ± 1.36 [a]	38.0 ± 2.37 [a]	38.8 ± 4.39 [b]
4 Salmon crumble + *Artemia*	0.48 ± 0.069 [a]	36.52 ± 1.30 [b]	77.6 ± 3.65 [bc]	38.5 ± 2.77 [b]
5 Salmon crumble	0.41 ± 0.047 [a]	34.21 ± 0.97 [a]	51.2 ± 4.71 [a]	37.0 ± 5.92 [b]

In terms of mean length of fish, the two treatments without co-feeding (3 and 5) resulted in significantly less growth (33.74 and 34.21 mm) than the control or co-feeding weaning treatments (44.19, 38.06 and 35.62 mm) (Table 2·3). However, in terms of mean weight, there was no significant difference between the co-feeding and non co-feeding treatments receiving salmon crumble. The Lansy ω3 diet with co-feeding was significantly superior to other weaning treatments in terms of weight gain ($P < 0.05$), but was still less than the control.

Only the control treatment had significantly more feeding fish and fewer deaths (77% feeders) than all other treatments, and treatment 2 (co-feeding Lansy) was better than all other weaning treatments (57.9% feeders) (Table 2·3).

Towards the end of the experiment, fingerlings were seen feeding off the bottom of the tank on freshly fallen food. No territorial or aggressive interactions were observed before, during or after food was offered. In the control treatment, food was only available below the bag. Cannibalism was never observed in the fingerling fish for the duration of the experiment.

2.3.2 Experiment 2.2. Comparison of Artemia, frozen zooplankton and formulated foods for weaning fingerlings of golden perch

Survival was significantly higher in the zooplankton fed control (82%) and the two gradual weaning treatments (62.4% for fish co-fed *Artemia* and 78% for fish co-fed zooplankton) (Table 2·4). The final weights were also significantly different between treatments, with the control treatment weighing significantly less (0.43 g) than any of the other treatments (1.11-1.57 g). The abrupt weaning treatment produced more feeding fish (>1 g)(97.2% of survivors) than any other treatment, but there was no significant difference between the final size of fingerlings weaned abruptly and the zooplankton weaning treatment.

The percentages of starved and feeding fish for the various dietary régimes are shown in Table 2·4. ANOVA showed that the percentage of feeding fish in each diet treatment was significantly different ($P<0.001$ in all cases), except that there was no significant different in percentages of surviving starved or feeding fish between the abrupt weaning treatment and the zooplankton co-fed treatment. The outcome for percentage starved fish also showed significant differences among the diets.

Table 2·4. Mean (± SE) weight, survival and weaning success of golden perch fed different weaning diets. Data are means of four replicates. Means with the same superscript are not significantly different (P<0.001). Mean starting weight was 0.16 ± 0.0097 g.

Diet	Weight (g)	Survival(%)	Feeding(%)	Starved(%)
Zooplankton (control)	0.43 ±0.23 [a]	82.0[a]	7.3 [a]	92.7 [c]
Artemia and crumble	1.11±1.07 [b]	62.4[a]	40.5 [b]	59.5 [b]
Zooplankton and crumble	1.57±0.55[c]	78.0 [a]	93.0 [c]	7.0 [a]
Abrupt crumble	1.57±0.62 [c]	13.3 [b]	97.2 [c]	2.8 [a]

2.3.3 Experiment 2.3. Weaning golden perch fingerlings using zooplankton and commercially available seafood products

Experiment 2.3. Trial 1.

Survival in all treatments except the control (86.3%) and roe treatment (26.7%) was low (<9%) (Table 2·5). The pilchard plus zooplankton treatment had no survivors in all three replicates so was not included in analysis. Survival of fish in the control was significantly higher ($P<0.001$) than all the other treatments, and survival in the roe treatment was significantly higher ($P<0.001$) than in all other treatments. Survival when compared to predicted survival (the control) was significantly different between each of the treatments except that the scallop treatment (5%) was not significantly different from the pilchard (6.6%) or the prawn (8.6%) treatments (Table 2·6).

The final weights and lengths of fish in the prawn (1.7 g, 42.8 mm), roe (1.3 g, 38.9 mm) and control (1.8 g, 42.8 mm) treatments were similar, while fish fed scallop (0.86 g, 34.7mm) weighed significantly less ($P<0.05$) and were significantly shorter ($P<0.01$) than those in the other treatments (Table 2·7).

The behaviour of the fish in response to the blocks of frozen feed differed between treatments. The zooplankton blocks dissolved slowly, and a stream of zooplankton fell through the mesh towards the tank bottom. After two days, more than half the fish in each tank were positioning themselves under the bag and feeding on the melting zooplankton. After six days all fish in the control tanks rushed to the bag as soon as it was placed in the tank. In the pilchard plus zooplankton treatment, the blocks initially fell apart and after two days about half of the fish were swimming up to the bottom of the bag. At six days, when the blocks were 100% pilchard, the fingerlings were positioning themselves at the base of the bag but the block stuck together and did not fall through the mesh. The bag had to be shaken for the pilchard to fall through, which frightened the perch to the bottom of the tank. The response to the pilchard only treatment was very poor. Fish in only one tank approached the feeding bag after two days and none did so after six days. The 100% pilchard blocks did not fall apart at all. A little feeding was observed when bags were shaken to release the minced pilchard, but generally all the fish in the tanks stayed at the bottom. The response to the prawn and scallop blocks was good, with about one third of fish swimming up to the bag

after two days, and about 75% doing so after six days. The blocks fell apart, although not as completely as the zooplankton blocks. Roe blocks melted and fell apart easily, and the feeding response was very similar to that with the zooplankton blocks.

Throughout the weaning period all the blocks of frozen food (except pilchard) melted and broke up well, sinking to the bottom of the tank. However, at the end of weaning period only fish in the zooplankton and roe treatments were responding vigorously to crumble sprinkled over the feeding area in the tank, with many feeding at the surface. In the other treatments, fish showed more subdued feeding activity, and stayed in mid-water or at the bottom, and at the end of the weaning period there was no feeding activity at all in both pilchard treatments. At the end of the experiment, all weaned fish were feeding at or near the surface.

Experiment 2.3. Trial 2.

Survival in all treatments was low but was comparatively better than in Trial 1 (Tables 2·5 and 2·6). Survival in the control treatment (48%) was much lower than the usual weaning rate of about 80%, and the number of survivors that appeared starved was also higher than in the previous trial. Survival of most treatments was higher than in Trial one, the highest being in the mussel treatment at 22.7 % and the fish treatment at 15.7%.

The weight of the control fish (0.28 g) was significantly ($P< 0.01$) heavier than that in all the treatments except prawn (0.2 g) and squid fed treatments (0.24 g)(Tables 2·5 and 2·7). Final weight and standard length values were higher for golden perch that successfully weaned using squid as the co-feeding diet when compared to all other treatments. There was no significant difference between golden perch weaned using squid or controls in both weight ($P<0.05$) and standard length ($P<0.01$). There were significant differences between the pilchard, mussel and prawn treatments in terms of final length but not weight.

At the end of the weaning treatments only control fish and a small number of mussel co-fed fish rose to food sprinkled on the surface but most stayed in mid-water and fed as the food sank. The survivors from all treatments did not appear to feed off the bottom after the food had sunk, whereas the control fish fed at all depths of the tanks.

Table 2·5. Mean (±SE) percentage survival and final weights of juvenile golden perch weaned using alternative fresh foods to zooplankton in Trial 1 and Trial 2. Different superscripts indicate significant differences between means (P<0.05).

		Weight for treatment	SL for treatment	Survival (%)	% starved of survivors
Trial 1	Control	1.83 ± 0.063 ^{c}	42.87 ± 0.563 ^{b}	86.3	6.56
	Pilchard	0.615 (n=2)	31.89 (n=2)	<1	-
	Prawn	1.57 ± 0.22 b^{c}	40.94 ± 1.341 ^{b}	8.6	15.38
	Roe	1.34 ± 0.088 ^{b}	40.28 ± 0.685 ^{ab}	28.7	34.88
	Scallop	0.93 ± 0.168 ^{b}	35.21 ± 1.136 ^{a}	5.0	26.67
	Pilchard + zooplankton	-	-	0	-
Trial 2.	Control	0.277 ± 0.012 ^{c}	22.56 ± 0.247 ^{c}	48.54	53.7
	Pilchard	0.166 ± 0.008 ^{a}	19.55 ± 0.259 ^{a}	15.7	48.9
	Prawn	0.205 ± 0.0246 ^{bc}	21.28 ± 0.688 ^{abc}	5.84	35.3
	Mussel	0.174 ± 0.0057 ^{ab}	19.82 ± 0.175 ^{ab}	22.73	16.9
	Squid	0.241 ± 0.028 ^{bc}	21.37 ± 0.687 ^{bc}	4.44	23.5

Table 2·6. Predicted proportions of survivors and results of pairwise comparisons between control and other treatments. Different superscripts indicate significant differences between means (P<0.001)

	Diet	Proportion of survivors	GLM estimate
Trial 1	Control	0.8636 [d]	Reference
	Pilchard	0.0066 [a]	-6.85
	Prawn	0.0864 [b]	-4.204
	Roe	0.2864 [c]	-2.759
	Scallop	0.0499 [ab]	-4.793
Trial 2	Control	0.4854 [d]	Reference
	Pilchard	0.1570 [b]	-1.674
	Prawn	0.0584 [ab]	-2.754
	Mussel	0.2273 [a]	-1.205
	Squid	0.0444 [ab]	-3.058

Table 2·7. The analysis of standard length and weight showed a significant weaning diet effect on growth. Different superscripts indicate significant differences within columns between means at the indicated P levels.

	Diet	Mean weight	Mean SL
Trial 1		(P<0.05)	(P<0.01)
	Control	1.824 ^{b}	42.85 ^{c}
	Prawn	1.698 ^{b}	41.75 ^{bc}
	Roe	1.317 ^{ab}	38.92 ^{b}
	Scallop	0.862 ^{a}	34.74 ^{a}
	LSD 5%	0.5883	3.811
Trial 2		(P<0.05)	(P<0.01)
	Control	0.2724 ^{c}	22.46 ^{c}
	Pilchard	0.1634 ^{a}	19.34 ^{a}
	Prawn2	0.2373 ^{abc}	21.75 ^{bc}
	Mussel	0.1781 ^{ab}	20.02 ^{ab}
	Squid	0.2230 ^{bc}	21.29 ^{bc}
	LSD 5%	0.07203	1.718

2.4 Discussion

2.4.1 Experiment 2.1. Weaning golden perch onto formulated diets using abrupt weaning, and co-feeding Artemia.

In this study, growth and survival of golden perch fingerlings were significantly higher overall in co-fed treatments than in non co-fed treatments. The *Artemia* diet was significantly better than the formulated diets in both survival and growth. This result is in contrast to that reported for marine fish species, which tend to grow and survive significantly better when co-fed live and formulated diets (Barnabe, 1991; Rosenlund *et al.*, 1997). Larvae of the Australian Macquarie perch, *Macquaria australasica*, showed better growth and survival when co-fed *Artemia* and formulated diets than on formulated diets alone (Sheikh-Eldin *et al.*, 1997). In Macquarie perch, a diet of enriched *Artemia* nauplii supported better growth and survival than non-enriched nauplii (Sheikh-Eldin *et al.*, 1997). In our experiment, enriching *Artemia* may have improved growth and survival in co-fed treatments of golden perch. However, as fish in these treatments were only co-fed for 10 days and were then fed entirely on formulated diets for 55 days, the observed differences in growth and survival may have been due to the formulations of the diets, rather than the quality and nutritional value of the *Artemia* offered early in the experiment.

Size of the fish at weaning is also an important factor in weaning success. Weaning in barramundi, *Lates calcarifer*, is most efficient at 17 mm TL, when they are at the transition stage between taking zooplanktonic food and benthic/demersal food items (Barlow and Rodgers, 1993). Smaller, planktivorous barramundi fry do not wean as well. Juvenile golden perch also have a developmental stage at which food types change, although this is related to gape size rather than a change in behaviour (Arumugam, 1986b; Arumugam and Geddes, 1988). Golden perch fingerlings of 25-30 mm TL weaned readily onto diet particles of 0.6 mm in this experiment. However, production of golden perch fingerlings for recreational fisheries enhancement requires 50 mm TL fingerlings. Production of golden perch fingerlings for weaning (for aquaculture) could be integrated into current production of for recreational fisheries.

Growth and survival of golden perch was better in co-feeding treatments than abrupt weaning treatments, in contrast to other Australian fish species such as silver perch (Rowland and Bryant, 1994), jade perch, *Scortum barcoo* (personal observation), and barramundi (Juario *et al.*, 1991; Barlow and Rodgers, 1993), that readily consume formulated diets. However, the comparatively high market value of golden perch may offset the additional expense involved in weaning.

This study demonstrated that golden perch fingerlings can be successfully weaned by co-feeding fresh food (*Artemia*) and formulated diets. The poor performance on formulated diets suggests that the diets used are nutritionally inadequate, difficult to assimilate, or lack palatability. Most of the formulated diets are formulated for marine, temperate fish, which may have different nutritional requirements to golden perch. By demonstrating that golden perch can be weaned successfully onto formulated foods, an essential requirement for large-scale production is now available.

2.4.2 Experiment 2.2. Comparison of Artemia, frozen zooplankton and formulated foods for weaning fingerlings of golden perch.

The zooplankton weaning treatment was superior to the *Artemia* weaning treatment, producing more feeding fish and fewer starved, and giving a higher mean weight. *Artemia* nauplii are nutritionally incomplete unless nutritionally enriched prior to use (Sheikh-Eldin *et al.*, 1997). Pond reared zooplankton may provide nutrients absent in *Artemia* nauplii (Dabrowski and Rusiecki, 1983; Coutteau and Sorgeloos, 1997). Colour can be important in diet acceptance by fish (Masterson and Garling, 1986). The high proportion of starved fish in the *Artemia* weaning treatment compared to zooplankton weaned fish could be explained by a longer time to accept the crumble diets (Lansy and salmon crumble), possibly due to the colour difference, as colour and contrast are important in acceptance of diets by fish (Ginetz and Larkin, 1973; Masterson and Garling, 1986; Hatanaka, 1997; Tamazouzt *et al.*, 2000). More rapid sinking crumbles may have limited the time that food was available to some fish. It is not thought that particle size or formulated food compared to the live food particle

size is an issue in this case, as golden perch ingest the largest food particles available, (Arumugam and Geddes, 1987) and the crumbles were uniform 0.6 mm size.

Although survival was significantly lower in the abrupt weaning treatment, the success of a small number of fish in that treatment suggests that gradual weaning is not essential for every fish in a population to wean. Our results indicate that gradual weaning is essential for large-scale production of a large proportion of golden perch that feed and grow well. The control (zooplankton) treatment produced a high survival rate, but a very high percentage of starved and a low mean weight. This suggests that they were malnourished, but that they were eating enough to survive. Zooplankton is susceptible to variations in nutritional value (Kubitza and Lovshin, 1999), and freezing may damage cells increasing leaching, so it is possible that the frozen zooplankton supplied was nutritionally inadequate.

The high survival rate (78%) in the gradual weaning process could probably be improved by refinements in technique and methodology. For example, determining optimal density; food delivery; feeding frequency; water temperature; tank cleaning and light régimes could improve weaning success. Weaning golden perch does not require labour intensive grading and sorting necessary for more cannibalistic carnivorous species such as barramundi (Maneewong, 1987). Improved survival of fingerlings, and managing nursery ponds to retain juvenile golden perch on formulated food, will be essential for development of a viable golden perch grow out industry. The results of this study will help achieve this objective.

2.4.3 Experiment 2.3. Weaning golden perch fingerlings using zooplankton and commercially available seafood products.

The commercially available seafood products trialed as alternative weaning diets to zooplankton were largely unsuccessful. Minced fish has been used as a supplement or as a fish food for many species of fish (Tay and Seow, 1974; Moura *et al.*, 2000), and was eaten by the juvenile golden perch. Also, as all diets except roe had to be washed through the sieves, it is possible that much of the nutritional and organoleptic properties of the minced flesh were lost. Although use of attractants in weaning diets is not always effective (Koskela *et al.*, 1991; Moura *et al.*, 2000), amino acids and

krill extracts can be highly effective feed attractants (Oikawa and March, 1996; Zhang *et al.*, 2002). Feeding stimulants can improve growth rates even when feed intake rates are the same, due to improved nutrient utilisation (Higuera, 2001). Thus, reduced quantity of stimulants in the alternative weaning foods may have resulted in substantially decreased nutrient absorption, even if food intake was adequate. Additionally, the difference in food colour may influenced the success of the weaning. Colour is an important determinant on feeding in fish (Ginetz and Larkin, 1973; Masterson and Garling, 1986), and the alternatives to the brown zooplankton were generally white in colour, except the yellow roes, multicoloured mussel and brown crumble.

The abrupt weaning on to the seafood alternatives from plankton may also have had a significant effect on the failure of transition to formulated diets, although the fish did appear to transfer well on to most of the seafood diets.

The survival rate of the control treatments illustrates the success of using zooplankton as a primary method of weaning. The behaviour of the zooplankton block, which melts slowly and enhances development of a strong feeding response, may in part explain the success of this method.

The poorer survival of the control group in the second treatment may indicate that smaller fish have a poorer weaning ability than larger fish. Larger fish have a greater capacity to survive the weaning process due to more stored reserves (Anderson, 1974; Flickinger *et al.*, 1975). It is also possible that this particular batch of fish had poorer weaning attributes. Variability in weaning success with differing batches of fingerlings has been found with Murray cod (Ingram, pers. comm. 2004), Eurasian perch (Babiak *et al.*, 2004) and sleepy cod (Mosig, 2002). However, the poor overall survivals of the various weaning alternatives still indicate that golden perch appear to be highly specific in their requirements for a successful weaning régime. Use of *Artemia* or zooplankton offers a relatively cheap and highly successful method of weaning.

The results of this trial suggested that initial size of fish is important at weaning, as the smaller fish in Trial 2 did not survive or grow as well as the larger fish used in

Trial 1. Knowing the best size at which to wean golden perch will be important in developing optimum weaning strategies for this species.

2.5 Conclusion

The results of the above experiments indicate that golden perch require a period of co-feeding to produce successful weaning of a large number of fish. Pond-reared zooplankton appears to be a better weaning diet than *Artemia*, possibly due to the extra nutritional value in pond zooplankton compared to newly hatched *Artemia* nauplii. The differences in colour or taste of the co-feeding diet, compared to the formulated food, appear to strongly influence the success of weaning, as indicated by the lower success of *Artemia* or washed out seafood compared to zooplankton. In Experiment 2.1, orange coloured Lansy diet also gave superior results when co-fed with *Artemia*. Zooplankton was frozen fresh and was also a similar colour to the salmon crumble.

The most successful treatments, and hence recommended procedure for weaning based on the results so far, are:

1. Use a gradual, co-feeding procedure to wean golden perch fingerlings. Replacement of the co-feeding diet with formulated food at the rate of 10% by volume per day gives good transition results.
2. Use frozen freshwater zooplankton if available, otherwise use *Artemia*, which is commercially available. Matching colour of formulated food and colour of transition diet may improve success rate. Other alternatives may be useable if they can be successfully fed to fish, but still retain their flavour and nutritive properties.
3. If supply of fingerlings and ethics permit, abrupt weaning may be applicable but it results in very low overall survival of fish that appear to grow equally as well as those weaned gradually.

The use of an expensive weaning diet (e.g. Lansy) for golden perch fry might produce slightly better growth in the end but was not found practical or necessary to produce acceptable results. The development of a diet for golden perch will be an essential element in development of aquaculture of this species. Development of aquaculture

of other species such as Murray cod (*Maccullochella peelii*) has been preceded by intensive research into the nutritional requirements of this species (Gunasekera *et al.*, 1998; De Silva *et al.*, 2000; Gunasekera *et al.*, 2000; Abery *et al.*, 2002; De Silva *et al.*, 2002), which has undoubtedly assisted in the development of this industry which produced 34 tonnes in 2001/2002 from negligible production in 1996 (O'Sullivan and Kiley, 1997; O'Sullivan and Savage, 2004).

The lack of cannibalism observed in these initial experiments is a favourable attribute for aquaculture. Many carnivorous species of fish are highly cannibalistic as fingerlings and require constant grading and handling to minimise mortalities in intensive culture. Golden perch do not appear to require handling for this reason, which is a major benefit in a high labour cost environment like Australia.

A range of other parameters regarding weaning require investigation, including shortening the weaning period, size of fingerlings at weaning, and effects of light on weaning. Abiotic factors can be as important as the obvious ones such as diet. Now that a successful, repeatable weaning technique has been developed, the effect of abiotic factors and temporal factors can be tested. These issues are assessed in the following Chapter 3.

Chapter 3
Further refinements of weaning techniques-effects of transition period, size at weaning and light intensity on weaning success

3.1 General Introduction

Weaning of juvenile fish onto formulated food has been pursued for many species of fish to ensure optimum nutrition and cheap or convenient availability of food without the expense of culture or handling of live or natural foods (Kubitza and Lovshin, 1999). The weaning process developed for golden perch in Chapter 2, using frozen zooplankton, was devised on the principle of keeping the process simple and using a 10 day process so that arithmetic was easy. The 10% volumetric increments were arrived at to make the transition from zooplankton to formulated food gradual. Observations of golden perch fingerlings during the co-feeding phase suggested that it might be possible to reduce the number of dilutions and thus reduce the number of days of co-feeding. The fish appeared to feed non-selectively on the crumble zooplankton mix. The experiments in Chapter 2 also indicated that a proportion of fish did not need a transition phase at all, but that this number was small (about 13%).

The transition period from live or fresh foods to completely formulated foods appears to be species-specific. For example, white bass (*Morone chrysops*) were weaned onto a dry crumble diet over a 14 day period (Denson and Smith, 1996), using a mix of frozen and live *Artemia*, zooplankton flakes and dry crumbles. North American perch (*Perca flavescens*) require training onto formulated food for 19-51 days (Malison and Held, 1992). One aim of shortening the weaning period is to reduce the costs of fresh food and reduce the time that fish are held in tanks. Many studies have shown that fish grow faster in cages or ponds than in tanks (Rowland *et al.*, 2004), so shortening the time that fish are held in tanks by using an abbreviated weaning process should have productivity benefits.

This Chapter reports on three experiments aimed to fine tune the weaning techniques for golden perch and, specifically, to determine the effect of transition period, size at weaning and light intensity on weaning success. The aim of Experiment 3.1 was to determine whether juvenile golden perch, generally accepted as being difficult to train onto formulated food (Anderson, 1986; Fallu and Mosig, 1994), could be trained to accept formulated food in a shorter time than the 10-day period previously used (Chapter 2). The advantage of this in commercial culture is that fingerlings would be held for a shorter period in the slow growth tank environment before being transferred to the faster growth cage or pond environment. Additionally, improvements in survivorship, the proportion of fish weaned, and growth in the weaning phase over the previous best practice were sought. A secondary aim was to elucidate whether a shorter period of holding fish after the weaning period could be used to determine the rate of weaning using a subjective method, to avoid the ethical issue of holding fish which did not make the transition, for longer than necessary. Determining the size at which juvenile fish can be weaned from live or fresh food across to formulated foods is essential in developing effective techniques for aquaculture. Many species including golden perch have a transition phase between larval and fry feeding behaviour at which food preferences change, usually at metamorphosis of the larval fish due to its increasing ability to select, pursue and ingest prey (Arumugam and Geddes, 1987; 1988; Kestemont *et al.*, 1996). This stage may be advantageous for weaning, as the fish may be more receptive to learnt cues rather than inbuilt reflex reactions to movement or smell. Barramundi is one species for which early weaning strategies focussed on the time when behaviour changed from actively pursuing prey to ambush, when they are 18-20 mm in length (Barlow *et al.*, 1993). Barramundi are generally weaned at about this size (Tucker, 2001).

Experiment 3.2 was designed to assess whether size of hatchery produced fingerlings at weaning plays an important role in weaning success. Most studies on weaning have focussed on larval weaning (fish less than 1 g in weight) (Gennari *et al.*, 1994; Denson and Smith, 1996; Næss *et al.*, 2001). There are a few studies which have investigated weaning of fingerling fish (Malison and Held, 1992; Kestemont *et al.*, 1996). Semi-intensive rearing has been found to be a more efficient fingerling production method for some species and for these, determining weaning size is

essential so that fish can be harvested at the optimal weaning size (Kestemont *et al.*, 1996; Næss *et al.*, 2001; Ljunggren *et al.*, 2003).

Weaning procedures for intensively cultured Australian fish differ between species. Larger size (83.3 mm, 6.0 g) Murray Cod (*M. peelii*) wean and grow faster than smaller ones (35.6 mm, 0.6 g), due to the larger mouth gape allowing ingestion of more food of varying sizes (Ingram, 2000). In silver perch, no weaning differences were detected between graded groups (1.5 and 2.99 g) cultured in tanks, suggesting that growth in silver perch remains relatively similar if size uniformity is maintained (Barki *et al.*, 2000).

Larger fish fingerlings may wean more successfully than smaller ones, due to their greater ability to sustain themselves over the transition period and greater ability to capture food particles (Anderson, 1974; Flickinger *et al.*, 1975; Otteraa *et al.*, 1994). However, small (16.9 mm) perch (*Perca flavescens*) weaned faster and grew to a larger ultimate size than larger (32.5 or 42.6 mm) fingerlings (Malison and Held, 1992) suggesting that in some species weaning at a smaller size has benefits beyond faster growth at an early age. Best size for weaning is therefore species-specific.

Initial attempts at weaning golden perch used fingerlings (18-31 mm TL) available from commercial hatcheries, which are primarily produced for restocking impoundments for recreational fisheries. These fingerlings are usually sold at about 50 mm TL. Many commercial hatcheries have a two stage fingerling production cycle where, after fingerlings have eaten all the food in one pond, they are moved to another pond. If golden perch can be weaned at a small size, grow out ponds could be stocked earlier. Hatcheries could increase production efficiencies due to shortened nursery rearing times and decreased attrition due to predation. As size at weaning may influence the ultimate size to which a fish grows (Malison and Held, 1992), it is important to determine if size at weaning has any effect on growth in golden perch.

Experiment 3.3 aimed to elucidate effects of one environmental factor, light intensity, on weaning success in golden perch. Again, this was designed to determine optimum environmental conditions in which to conduct weaning. The influence of environmental factors on production of essentially non-domesticated animals is of

great importance in developing techniques for their culture. In natural conditions, golden perch adults prefer deep (>6 m) water and shelter, but these patterns of distribution change at night time (Crook *et al.*, 2001), indicating behavioural changes associated with light intensity. Providing environmental conditions conducive to growth and health are critical for success of intensive aquaculture. Tank colour, which affects the light intensity within the tank, is also of importance in fish production (Denson and Smith, 1996; Hatanaka, 1997; Tamazouzt *et al.*, 2000).

It is not clear whether light intensity alone influences fish growth rate, because receptivity of fish to light differs profoundly with species and age (development) of the fish (Boeuf and le Bail, 1999). Photoperiod is more important in growth of fish than light intensity (Boeuf and le Bail, 1999). Very few studies have documented effects of light intensity on juvenile fish as opposed to larvae. In salmon fry, growth is better but mortality higher, at 700 lux than at 200, 50 or 10 lux, indicating a stress effect of high light intensity (Wallace and Kolbeinshavn, 1988). Other experiments (Oppedal *et al.*, 1977; Huse *et al.*, 1990) showed slight effects but these could have been indirectly influenced by rearing environments.

Some larval fish are able to locate food in dark environments. Striped bass (*Morone saxatilis*) use mechano – or chemo-sensory strategies to locate food in the dark (Chesney, 1989). Survival and growth of a number of fish species are significantly improved in low light or dark conditions (Wallace and Kolbeinshavn, 1988; Hatanaka, 1997; Baras *et al.*, 1998; Crook *et al.*, 2001; Trippel and Neil, 2003) (Table 3·1). However, complete darkness reduces food capture ability in some fish (Rubio *et al.*, 2003). Poor growth and survival has also been recorded for Eurasian perch larvae in dark conditions (Tamazouzt *et al.*, 2000). Chesney (1989) postulated that light intensity has a strong influence on foraging of juvenile fishes because of the distance at which they perceive prey.

As the natural habitat of golden perch includes highly turbid waters, it was expected that light intensity may be an important factor in growth and survival of golden perch being weaned in tanks. The aim of this experiment was to elucidate whether golden perch fingerlings would wean successfully in conditions of bright light, low light or complete darkness.

Table 3·1. Effects of light intensity on juvenile fish of various species.

Species of fish	Effect of light	Source
Vundu catfish *(Heterobranchus longifilis)*	Reduced cannibalism and increased growth when fed at night	(Baras *et al.*, 1998)
Striped bass *(Morone saxatilis)*	Reduced light reduced growth and forage rates	(Chesney, 1989)
Golden perch *(Macquaria ambigua)*	Associate with structure and deep water when light, in darkness move around and away from structure.	(Crook *et al.*, 2001)
Tiger puffer *(Takifugu rubripus)*	High light intensity and/or light coloured tanks reduced growth	(Hatanaka, 1997)
European sea bass *(Dicentrarchus labrax)*	Food catching ability in the dark78.6%, compared to 93.5% in diurnally fed fish	(Rubio *et al.*, 2003)
European perch *(Perca fluviatilis)*	Reduced growth and survival in low light	(Tamazouzt *et al.*, 2000)
Haddock *(Melanogrammus aeglefinus)*	Reduced light intensity increase growth by 11%	(Trippel and Neil, 2003)
Arctic charr *(Salvelinus alpinus)*	Mortality reduced, growth increased at low light intensity	(Wallace and Kolbeinshavn, 1988)

The ultimate aim of this series of experiments was to determine some of the environmental conditions which would be conducive to weaning of golden perch in tanks, to further develop protocols for weaning of golden perch in intensive systems.

3.2 Materials and Methods

3.2.1 Experiment 3.1. Effect of transition period length on weaning of golden perch

Golden perch obtained from a commercial hatchery were quarantined, graded through a 4.9 mm width bar grader (Mean weight ± SE 1.49 ± 0.33 g, SL ± SE 39.7 ± 2.77 mm) and stocked at 100 per 100 L tank (section 2.2.1.2 for details). Water temperature was 25° ± 2°C throughout the course of this experiment.

Light intensity averaged 1.85 lux during daylight hours (06:00-18:00), although when lights were turned on for cleaning light intensity rose to about 7 lux for 40-60 minutes while tanks were cleaned and mortalities etc. removed. Refer to Section 3.2.3 for clarification of light readings. Lights were turned off after cleaning, a minimum of two hours before the next feed. Crumble fed was 1mm Pivot salmon crumble (55% protein, 15.4% fat, 4.1% fibre, 11.8% ash and 13.7% carbohydrate).

Four weaning treatments were applied, including a control, in four randomly assigned replicate tanks. Each treatment had a six-day acclimation period when they were fed on frozen zooplankton, after which weaning commenced (Table 3·2).

- The control treatment was nine days of transition from zooplankton to crumble diet, with 10% by volume increase in proportion of crumble mixed with zooplankton every day as described in Chapter 2.2.2.
- Treatment 1 was two days fed at 70% zooplankton (P), 30% crumble (C), followed by two days at 50% P:50% C then two days at 20% P:80% C, then on to 100% C.
- Treatment 2 was six days fed a 50 P:50 C mix.
- Treatment 3 was three days fed on 50 P:50 C mix.
- An extra treatment (Treatment 4) with only two replicates (due to tank number limitations) was identical to the control except feeding only twice per day (08:00 and 16:00h) instead of three times.

Table 3·2. Experimental layout of abbreviated weaning trials. Numerical values are percentage of crumble diet mixed with zooplankton for weaning.

	Day of weaning									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Control	10	20	30	40	50	60	70	80	90	100
Treatment 1	30	30	50	50	80	80	100	100	100	100
Treatment 2	50	50	50	50	50	50	100	100	100	100
Treatment 3	50	50	50	100	100	100	100	100	100	100
Treatment 4 (2 reps)	Same as control but only two feeds per day									

Ten days after the weaning period was complete (a total of twenty days after weaning commenced); all fish were weighed after feeding (to allow identification of feeding fish). Measurements of length were not taken due to the handling involved resulting in a high mortality of fish of this size. Weaning success was judged by the survival rate and also by a subjective condition index (feeding or starved fish). Golden perch with grossly distended abdomens were considered to be feeding, while those that had sunken abdomens were considered not to be weaned.

Average weight at the end of the experiment was tested using analysis of variance for a completely randomised design. Survival and starvation rate between treatments was compared using ANOVA on arcsine transformed data. Genstat 6.1 was used for these analyses.

One control treatment replicate became infected with white spot (*Ichthyophthirius multifiliis*) during the experiment so was not included in the final analysis. For ethical reasons the trial was terminated ten days after the transition process was complete, so that fish which had not made the transition could be euthanised rather than starve to death. The experiment was approved by the Far North Queensland Animal Ethics Committee, approval number FNQ- 02-01.

3.2.2 Experiment 3.2. Weaning of golden perch fingerlings of different sizes

Golden perch fingerlings were obtained from a commercial hatchery and transported to FFAC. After prophylactic treatments and a 4 day recovery period, fish were graded through a bar grader to exclude extremes of small (<2 mm bar gap) and large (>4.2 mm bar gap). Fish for the ungraded control treatment were removed after this grading. The remaining fish were split into three size classes (small, medium and large), using a bar grader (Table 3·5).

One hundred fish of each class and the ungraded control were put into 100 L round tanks in a subdued light environment (day light intensity 1.818 ± 0.124 lux, night 0.001076 ± 0 lux, as per explanation of light readings in Section 3.2.3). Tanks were provided with aeration and flow through bore water at 26° C. There were four replicates of each treatment. Fish were weaned according to methods described in Chapter 2. Mortalities were removed and replaced during the acclimation period of one week when fish were fed frozen zooplankton to excess. Once weaning commenced no replacement of mortalities were made. The experiment ran for 31 days after weaning commenced, and finished when there were no mortalities over a ten day period after weaning. Fish were fed to excess after the weaning process was completed. Initially, a random sample of 20 fish was taken from each tank for weight and length measurements. The smallest size class were not weighed due to limitations of equipment and because handling of such small fish (blotting) results in high mortality. At the end of the trial, every fish from every tank was measured and weighed. Specific growth rate was calculated using the equation:

$$SGR = \frac{\text{ln final weight - ln initial weight}}{\text{time in days}} \times 100$$

(Busacker *et al.*, 1990), to determine if fish of different sizes but the same age had different growth rates. The experiment was approved by the Far North Queensland Animal Ethics Committee, approval number FNQ- 06-00.

All data was analysed using Genstat 6.1. Differences in Specific Growth Rate (SGR), weight and standard length between treatments were tested using randomised block

design ANOVA. Differences in survival on arcsine transformed data were tested using ANOVA. Coefficient of variation for weight in each treatment was plotted as a regression to compare whether small fish growth was more variable than large fish.

3.2.3 Experiment 3.3. Effect of Light on Weaning

Fingerling golden perch were air freighted to FFAC from a commercial fish hatchery. They were acclimated to local water, given prophylactic formalin and salt treatments, and fed frozen zooplankton for 5 days before transfer into experimental tanks. Tank set-up was detailed in section 2.2.1.2. Frozen zooplankton, sieved to between 350 μm and 1.18 mm, was primarily *Moina micrura*, with less than 10% by count of *Daphnia carinata*, cyclopoid and calanoid copepods,.

Three light intensity treatments were used. Three adjacent tanks in the tank array were curtained off with black plastic (0.2 mm concrete underlay) resulting in two blocks with three replicate tanks of each treatment. In the high light intensity treatment, a 60 watt 320 lumen daylight simulation globe was suspended 650 mm above each tank, attached to a timer for a 12:12 day night cycle from 06:00 to 18:00h. Tanks in the ambient light treatment received diffuse daylight from windows either side of the building, but never direct sunlight or artificial light. Tanks in the dark treatment remained dark at all times except for cleaning when a 40 watt red globe was turned on for 10 – 15 minutes (intensity of light 0.00215 lux). Light intensity was monitored for each blocked treatment using a stowaway light intensity logger (Onset Computer Corporation Australia) which reads light in lumens/square foot. This was converted to lux using the equation lumens sq. ft=10.76396 lux. Light readings are presented in Table 3·3. This meter is calibrated on an incandescent light source, and reads about a factor of six low (compared to a standard photographic light meter) when measuring indirect sunlight. A correction factor is indicated in Table 3·3.

Table 3·3. Mean (± SE) light intensity readings for treatments. Means of light intensity in lux from 06:00 to 17:59 (day) and 18:00 to 05:59(night).

	Day	Night	Correction (day)
Dark	0.00965± 0.00275	0.00108±0	0.058
Ambient	1.79±0.081	0.0145±0.0043	10.74
Light	73.64±0.55	0.00108±0	441.8

Fingerlings were graded between 3.5 mm and 4.2 mm bar gap in a bar grader for the experiment. A random sample of 50 graded fish was sampled and measured (25.9 ± 3.74 mm; 0.34 ± 0.1 g) before 100 fish were distributed to each tank. Weaning was started after an acclimation period of three days, after which normal feeding behaviour had resumed. Fish were weaned using the zooplankton co-feeding method (Herbert and Graham, 2004). Fish were fed crumble for 24 days post-weaning and on day 25 post-weaning were collected, sedated using Aqui-s (isoeuganol) measured and weighed.

Water temperatures were monitored hourly using calibrated temperature loggers (Tinyview plus, Gemini Loggers) and ranged from 24.7 – 26.5° mean 25.39°C. A TPS 90 – FL water checker was used to check pH and oxygen levels twice a week.

As an estimate of condition, a condition factor of $K = wt/SL^3$ was used (Busacker *et al.*, 1990), multiplied by a scaling constant of 10^5. Additionally, a note was made of the subjective condition factor (section 3.1.2).

Final weight, length and condition factor were tested using a randomised block structure ANOVA. Pair wise comparisons of final weights were done for each treatment using Kolmogarov-Smirnov two sample tests. Probability level was <0.05 unless otherwise stated. All tests were done using Genstat 6.1 (Lawes Agricultural Trust, Rothamsted Experimental Station). The experiment was approved by the Far North Queensland Animal Ethics Committee, approval number FNQ-09-01.

3.3 Results

3.3.1 Experiment 3.1. Effect of transition period length on weaning of golden perch

There were no significant differences in mean final weight between all the treatments. There was also no significant difference in final weight between the control and Treatment 1 (70/50/20).

Survival in all treatments was equal, or close to 100% (Table 3·4). There was no significant difference between survival rates in all treatments (F= 0.55). Feeding observations and final weight were used to determine weaning success rather than survival. The condition factor (% starved) was highly significantly different for the two 50% treatments, then less different with the more gradual Treatment 1 (70-50-20%) and two feeds per day treatments. The control had the lowest number of starved fish (38.4%) and the heaviest mean weights (2.1± 0.9 g) compared to all other treatments. The two feeds per day treatment had the second lowest number of starved fish and end weight similar to the abbreviated weaning treatments. The similarity in mean weights of each treatment control and starved fish is similar, but the mean weights of feeding fish in the different treatments varies between treatments (Figure 3.1). Starved and feeding fish had very small standard errors, whereas the combined total mean had a high standard error.

Table 3·4. Mean (± SE) survival, final weight and percentage of non-feeding fish at end of experiment. Means with the same superscript were not significantly different at the 0.05 level.

Treatment	Final weight (g)	Survival (%)	% starved
Control	2.1 ± 0.965 ^{b}	99.67	38.40 ^{a}
50% three days	1.6 ± 0.825 ^{a}	100	60.82 ^{c}
50% six days	1.6 ± 0.988 ^{a}	99.67	64.01 ^{c}
70/50/20%	1.8 ± 0.987 ^{ab}	99.5	54.40 ^{bc}
2 feeds/day	1.7 ± 0.377 ^{a}	99.5	48.19 ^{ab}

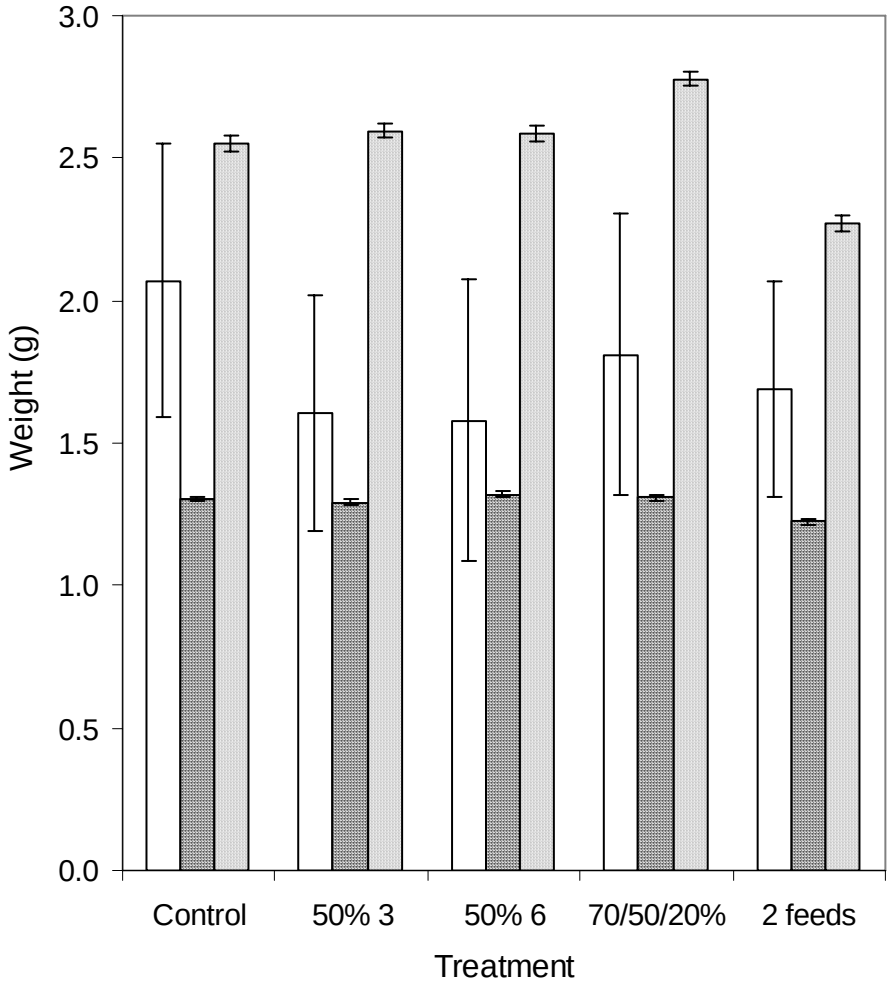

Figure 3·1. Mean weight (± SE) of golden perch in abbreviated weaning trials. Open boxes are means, bricked is starved fish, and stippled is feeding fish. 50% 3 is the 50% crumble/zooplankton mix over three days; 50% 6 is the 50% crumble/zooplankton mix over six days; 70/50/20% is the treatment with three different proportions of crumble offered during the weaning period.

3.3.2 Experiment 3.2. Weaning of golden perch fingerlings of different sizes

Survival was high in all treatments, and was not significantly different between treatments. All fish in all tanks appeared to behave similarly before, after and during meal times and also during cleaning. No aggressive interactions were observed between fish.

Average survival of fish and SGR for each treatment is shown in Table 3·5. There were no significant differences between ungraded and medium sized fish in any parameter measured. The small and large treatments differed from the medium and control groups ($P<0.05$) and each other (Table 3·5). SGR was significantly higher in fish weaned at the smallest size range than all treatments. The large fish had the lowest SGR and weight gain, with SGR of 3.55% and weight gain of 300%.

Coefficients of variation were high and are plotted in Figure 3-2. The controls had the highest variation, but there was a significant linear relationship from large fish to small fish, with small fish having higher CV than large ones.

Table 3·5. Mean (± SE) start size, survival, SGR and final size of golden perch weaned at different sizes. *calculated weight from regression of other weights of fish in this experiment

	Start weight (g)	Start SL {mm)	Survival (%)	Final weight (g)	Final SL(mm)	SGR (%)	% Increase in weight
Small	0.103* ± 0.00632	19.28 ± 0.173	96.2 [a]	0.43 ± 0.0237 [c]	25.57 ± 0.318 [c]	4.60 [c]	416
Medium	0.154 ± 0.0052	20.37 ± 0.168	99.7 [a]	0.529 ± 0.294 [b]	27.40 ± 0.698 [b]	3.98 [b]	343
Large	0.239 ± 0.00622	22.58 ± 0.191	91.6 [a]	0.717 ± 0.0184 [a]	30.52 ± 0.461 [a]	3.55 [a]	300
Control	0.153 ± 0.00663	20.58 ± 0.209	94 [a]	0.527 ± 0.0303 [b]	26.84 ± 0.380 [b]	3.99 [b]	344

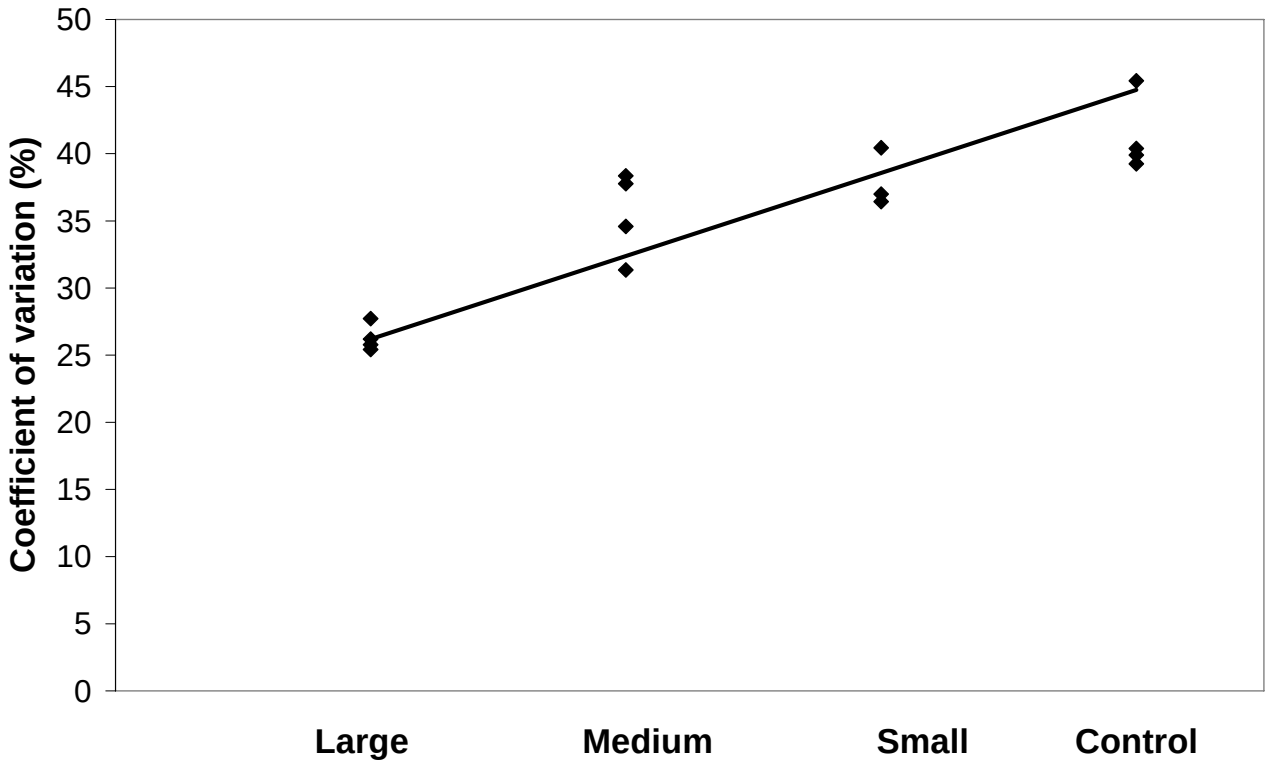

Figure 3·2. Coefficient of variation of golden perch weaned at different sizes (large, medium and small). The CV of large fish is lower than that of the small fish.

3.3.3 Experiment 3.3. Effect of Light On Weaning

Fish in the dark treatment had significantly lower growth, survival and condition than those in either ambient or light conditions. The fish in the ambient treatment had marginally higher, but significant differences in weight but not length, compared to fish in the light treatment (Table 3·6).

The high light intensity treatment had the best average survival rates, with all replicates having 98% survival or above. One of the ambient treatments had only 94% survival, although other replicates were >98%. The dark treatment averaged 80% survival, significantly lower than the other treatments.

Table 3·6. Mean (± SE) final weight, length, survival, condition index and starvation rates of golden perch weaned at three different light intensities; dark (0.00965 lux), ambient (1.79 lux) and light (73.64 lux).

	Weight (g)	SL (mm)	Survival (%)	Condition (wt/SL^3)	% Starved
Light	1.073 ± 0.013 [b]	35.18 ± 0.15 [b]	98.7±1.03 [b]	2.988 ± 0.010 [b]	0.17
Ambient	1.284 ± 0.017 [c]	36.40 ± 0.16 [b]	97.3 ± 2.16 [c]	3.452 ± 0.013 [c]	0.67
Dark	0.676 ± 0.018 [a]	29.85 ± 0.22 [a]	80 ± 6.07 [a]	2.135 ± 0.021 [a]	93.3
LSD	0.1084	1.692	5.38	0.2002	
F	0.014	0.011	0.006	0.019	

The condition factor for the fish in the dark treatment was also significantly poorer than those in the light and ambient treatments. However, the subjective condition factor (as told by the sunken abdomen) was far lower in the dark treatment (56 or 93% of survivors) than in the light (1 or 0.17%) or ambient treatments(4 or 0.67%).

3.4 Discussion.

3.4.1 Experiment 3.1. Effect of transition period length on weaning of golden perch

The higher rate of transition to weaning over a 10 day gradual weaning period demonstrated that under the conditions of this trial, golden perch require a time frame of more than six days, and a gradual transition, to wean successfully on to formulated food. A transition period of six days with gradual introduction of the formulated food only produced slightly more than half of the weaned fish than did the full 10-day process. The growth rate of the fish that did wean in the 70-50-20% treatment suggested that the gradual approach may be more important than the duration of the

weaning period. The similarity of sizes of fish that did not wean suggests that these fish which did not wean successfully stopped feeding at about the same time, possibly because food was not palatable to a proportion of fish, so all food was rejected from the onset of weaning. It could also indicate that from an early stage less aggressive individuals had less access to the reducing amount of natural food and so were not exposed to the transition diets, only to the formulated food, although interactive behaviour was not observed. This apparent need for a gradual series of co-feeding mixes, over an extended period, may be the reason why previous attempts at weaning golden perch have not succeeded, leading to the notion that golden perch are difficult to wean (Anderson, 1986; Fallu and Mosig, 1994).

The results of this experiment indicate the importance of developing techniques specific to a particular species. There are substantial differences in the techniques used to wean North American bass species (*Morone* spp.) depending on the purpose for which they are reared and the species (Anderson, 1974; Flickinger *et al.*, 1975; Bondari, 1983; Denson and Smith, 1996; Kubitza and Lovshin, 1999). Some species of fish make an abrupt transition to formulated food well; while others take weeks of almost continuous co-feeding. The results of this experiment give an indication of the fineness required in developing a weaning technique for any species of fish which does not wean easily. The finding that a gradual approach was far better than only using one dilution, suggests that any less gradual approach than the 10% increments would produce a poor result.

The use of condition as a method to assess success of weaning meets ethical standards by not subjecting fish to undue stresses. A similar subjective assessment was used to assess weaning of pikeperch and perch (Ljunggren *et al.*, 2003). This method permits valid results to be obtained without subjecting the animals to a prolonged death from starvation. The use of a subjective condition factor reduces the period of starvation to that which is no longer beyond repair when allowing assessment of weaning success accurately, and reduces handling of fish required for obtaining length measurements.

3.4.2 Experiment 3.2. Weaning of golden perch fingerlings of different sizes

Growth expressed as SGR or as percentage weight gain over the entire period was significantly higher in the smaller fish than the larger fish. This is to be expected as smaller fish have a higher growth potential than larger fish, as is exhibited in several other species (Jobling, 1983). For example, Atlantic halibut weaning trials produced SGR rates of 3.18 for small fish and only 2.38 for the larger size class (Næss *et al.*, 2001). Graded silver perch (*Bidyanus bidyanus*) had SGR of 2.2 using fish of 2 g, while larger fish (>2 g) had lower SGR of 1.95 (Barki *et al.*, 2000).

Disturbance and handling of fish has been found to significantly reduce growth (Mélard *et al.*, 1996a; Ogle *et al.*, 2001). In this experiment, daily cleaning may have had a deleterious impact on growth. SGR may also be suppressed by the presence of larger fish (Barki *et al.*, 2000), but in this experiment the growth of ungraded fish was not different to that of graded fish. This suggests that inhibition of growth due to size differentials did not occur in golden perch over the short period of this experiment. The lack of difference between the control and medium treatments suggests that, in the conditions of this trial, grading does not impact on growth rates of juvenile golden perch, and size variation does not impact on growth or weaning success.

Weaning success in yellow perch (*Perca flavescens*) (measured by survival) was not different at different sizes, but fish weaned at a smaller size grew to a larger ultimate size than those harvested and weaned at a larger size (Malison and Held, 1992). This has important implications for aquaculture. The potential for increased productivity of pond reared yellow perch fingerlings through a shorter pond phase (Malison and Held, 1992) could be realised in production of golden perch in Australia, which has essentially the same system of fingerling fish production. Earlier harvesting of fingerlings also allows higher survival of fingerlings due to lack of attrition through starvation, predation, and cannibalism (Malison and Held, 1992).

The higher CV of the small fish compared to large fish is to be expected. Growth potential of smaller fish is higher (Jobling, 1983) and it is likely that variations in growth potential could result in the higher CV observed. Variations in weight of

larger fish would have be much larger to produce a comparable CV. Disparities in the smaller fish could push CV higher more so than disparities in larger fish. The higher CV and SGR of the smaller fish may indicate that a proportion of smaller fish still have high growth potentials.

The results of this study indicate that smaller golden perch fingerlings do wean equally as well as larger ones, demonstrating the feasibility of weaning at an earlier age, which will fit in with current production practices. Weaning at a smaller size (and consequent shorter pond production cycles) could allow for more crops of fingerlings to be produced in zooplankton ponds over the 5-7 month production period of fish hatcheries in southern Queensland.

3.4.3 Experiment 3.3. Effect of Light on Weaning

Light is clearly important, although not essential, for golden perch juveniles to find food and ingest it. Although fish in the dark treatment had significantly lower survival and growth than those in the two treatments with light, the observation that fish kept in almost complete darkness grew and had a reasonable survival rate indicates that a proportion of golden perch juveniles can find food in darkness. This is probably an adaptation to the highly turbid environment that many golden perch live in. This study demonstrates that 70 – 80% of the fish that were in the dark could locate food without visual cues, suggesting that they used smell to locate food. Chesney (1989) found that striped bass used chemosensory strategies to find food in the dark or turbid waters; golden perch may also use this strategy. The results of the alternatives to zooplankton experiment (Section 2.3.3), when interpreted on the basis of these results, strongly suggest that olfactory qualities of the food are critical in weaning.

However, visual acuity also plays an important role as the overall growth rates and condition were significantly better in those treatments where golden perch were able to see food. The intensity of light influenced growth (as weight) significantly, contradicting the argument of Boeuf and Le Bail (1999) that light intensity was not important in stimulation of growth of fish. The slight effects of different light intensities could have been related to stress as the golden perch in the light treatment always stayed at the bottom of the tank even during feeding, whereas the fish in

ambient treatment rose to the surface at feeding and thus had longer exposure to the food as it was sinking and then on the bottom. The fish in the dark treatment may have had less time to eat the crumble food as it was sinking, because they did not appear to feed off the bottom, so would only have been able to eat the food as it was sinking.

High light intensities cause reduced growth and feeding response in several species of fish (Hilge and Steffens, 1996; Hatanaka, 1997) and appear to cause death of larval fish (Denson and Smith, 1996; Trippel and Neil, 2003). Conversely, striped bass and Eurasian perch grow faster in high light intensity (Chesney, 1989; Tamazouzt *et al.*, 2000). Even sight-dependent fish such as salmonids feed and grow better under low light conditions as high intensity light stresses them, reducing growth (Wallace and Kolbeinshavn, 1988). Light intensities of over 2000 lux were used in these experiments. As golden perch exhibit slightly better weight gain and condition in ambient light than in bright light, possibly bright conditions are stressful.

The differences between the effects of light on golden perch and other fish species emphasises the need for species-specific research to determine optimal conditions for their culture. The performance of golden perch at the low light intensity and the apparent lower stress suggest that low light intensity is best for this species of fish, but that some light is essential for them to grow (probably through feeding efficiency). It also has implications in pond aquaculture as if fish do prefer dim light they will stay at the deepest part of the pond if the water is clear. In turbid water they may be more likely to use the whole pond more and have less interactions with conspecifics. Avoidance of light by golden perch adults has been demonstrated by telemetry studies indicating that golden perch stay deeper than 6 metres at day but move around freely at night, in a clear water reservoir (Anonymous, 2003). This suggests that feeding of golden perch in aquaculture ponds at dusk and dawn will be the best time to feed the fish. It also suggests dim lighting conditions for tank-based production or experimental systems.

3.5 Conclusion

The gradual transition from natural foods to formulated food is critical in weaning golden perch. A minimum ten day transition period is essential, as it produces far higher survival and growth than any of the shorter treatments with fewer dilutions of foods. The results of the size at weaning trial indicate that small golden perch fingerlings wean equally as well as larger ones and provide an opportunity for hatcheries to shorten the pond phase to ease transport of fingerling fish, thus increasing production efficiency. Environmental conditions do play an important part in ensuring success of golden perch weaning. Provision of a dimly lit environment appears to improve growth rates marginally and influences behaviour of fish, which may improve growth rates over a longer period than this study.

Low light intensity, gradual weaning and weaning small fingerlings will increase success in weaning golden perch, which allows further exploration of growout possibilities in intensive culture systems.

Chapter 4
Growth of golden perch fingerlings in experimental tanks.

4.1 General introduction

This chapter reports findings of two experiments investigating effects of density and diet texture on weaned golden perch fingerlings. The density experiment (Experiment 4.1) aimed to validate the densities used in trials reported in the previous chapter. The comparison of dry and moist diets (Experiment 4.2) aimed to confirm the perception within industry that golden perch may prefer moist pellets, and communications from other researchers who suspected that texture of hard pellets was one factor impeding weaning golden perch on to formulated diets.

Density in aquaculture has major effects on behaviour, physiology and health of the fish (Table 4.1). It is of critical importance in intensive systems where filtration or water circulation must be matched with potential waste generation of fish biomass in the system. It is also important in pond or cage aquaculture where knowledge or understanding of loadings and carrying capacity are critical in maintaining stocks in a healthy, ethical and sustainable manner. Density, when applied to fish culture, is a rubbery term as there are no established criteria for what constitutes high and low density for fingerling fish. Density studies referred to below all compared 'high' densities with 'low' densities but the terms high and low density are only comparative within a particular set of conditions.

Behavioural effects of density can be either beneficial or detrimental in aquaculture. Some species, such as silver perch and Murray cod may develop hierarchies at 'low' densities which are broken down when they are kept at higher densities (Lovric, 2000; Harpaz *et al.*, 2001). Silver perch hierarchy establishment in a recirculating tank system resulted in gashes or holes in the flesh in the first few weeks, but at high densities there were less mortalities due to these aggressive interactions (Harpaz *et al.*,

2001). Growth of silver perch was found to be equal at several higher densities in another study on silver perch in cages and tanks, at densities of up to 200 fish (21 kg/m^3) in cages or flow through tanks (Rowland *et al.*, 2004). Decreased territorial and aggressive behaviour in Eurasian perch at high densities of fingerlings resulted in a 67% increase in growth (Mélard *et al.*, 1996a). Arctic charr and other salmonids grow significantly better at high densities due to suppression of aggressive spacing behaviour (Brännäs and Linnér, 2000).

Conversely, many studies indicate that in some species lower densities are beneficial and permit faster growth than high density culture (Hatanaka, 1997; Maragoudaki *et al.*, 1999; Anderson *et al.*, 2002; Jodun *et al.*, 2002; Saillant *et al.*, 2003) (Table 4·1). African eeltailed catfishes (*Clarias* spp.) cultured at high density have depressed growth (Oellermann and Hecht, 1998; Chude, 2001), although at low density aggressive interactions increase (Kaiser *et al.*, 1995). Some of these behaviours may be linked to out-of-phase feeding times in fish which are normally nocturnal (Baras *et al.*, 1998).

Another effect of density relates to chemical secretions of the animal, which can affect growth. This is well documented in the giant freshwater prawn *Macrobrachium rosenbergii* where dominant males restrict growth of other prawns by suppressing food conversion efficiency (New, 2002). High rearing densities can invoke immune responses in tilapia (*Tilapia mossambica*) (Henderson-Arzapalo and Stickney, 1982), and reduce immune capability in ayu (Iguchi *et al.*, 2003). High density can also either repress or enhance gene expression (Gornati *et al.*, 2004).

Table 4·1. Effect of density on selected fish species.

Species of fish	Density	Effect of density	Source
Bluegills *Lepomis macrochirus*	167, 334, 500, and 667 fish/m^3	Size was inversely related to stocking density. % weight gain and feed efficiency decreased as stocking density increased	(Anderson *et al.*, 2002)
African catfish *Clarias gariepinus*	one to three individuals/m^2	Growth rate reduced by as much as 75% when the density was increased	(Chude, 2001)
Atlantic sturgeon *Acipenser oxyrinchus*	3.6-10.9 kg/m^2	growth rate was inversely proportional to density	(Jodun *et al.*, 2002)
Sea bass *Dicentrarchus labrax*	10, 80 and 100 kg/m^3 of 50g fish	Population density has an effect at gene level by repressing or enhancing the expression of different genes.	(Gornati *et al.*, 2004)
Tiger puffer *Takifugu rubripus*	300-600 fish/m^3.	Total length, body length, body weight, and the normality of caudal fin (sign of aggressive interactions) higher in low density.	(Hatanaka, 1997)
Tilapia *Tilapia aurea* and *T. mossambica*	5, 10, 20, 30, 40, 50 and 60 fish/tank in 60 L of water	*T. mossambica* experienced much higher mortality and reduced growth due to an auto-immune reaction related to high stocking density	(Henderson-Arzapalo and Stickney, 1982)
African catfish *Clarias gariepinus*	1.2, 0.6 and 0.3 fish / cm^2	Highest aggression at medium density, no differences in growth	(Kaiser *et al.*, 1995)

Hybrid catfish *Heterobranchus longifilis* ♂ x *Clarias gariepinus*♀	250, 1000 and 2000 fish / m^3	Fish in the middle and higher density tanks grew significantly faster	(Oellermann and Hecht, 1998)
Red porgy *Pagrus pagrus*	500-L tanks at densities of either 50 or 100 fish (100 or 200 / m^3)	Fish stocked at low density and feeding freely grew better than high density fish	(Maragoudaki *et al.*, 1999)
Sea bass *Dicentrarchus labrax*	Various	No effect of rearing density was detected on sex ratio. Larvae reared at low density grew faster. During juvenile development, fish reared at high density grew faster	(Saillant *et al.*, 2003)

Behavioural and physiological responses to crowding are species-specific and may also be in part determined by the system in which the fish live, and the natural biorhythms of the fish, i.e. nocturnal vs. diurnal, lunar, etc.; e.g. Baras *et al*. (1998). The effect of density on golden perch in tanks requires investigation as it may give leads to whether hierarchical, behavioural or physiological factors will impact growth. It may also give important clues as to behavioural or physiological traits, which may be of importance in intensive systems. This chapter reports the results of two experiments aimed at determining growth rates of weaned golden perch fingerlings in tanks. Firstly, the effect of density on growth was tested to determine if social factors would influence growth, and secondly a moist diet was tested against equivalent dry diets to determine if texture was a determinant in feed acceptance. The aim of Experiment 4.1 was to determine if density of juvenile golden perch (in tanks) had a major impact on growth or survival.

The use of formulated feeds in aquaculture has long been researched as a means of improving nutrition, having consistency in food, and for ease of storage and handling (Marsden *et al.*, 1997). Texture of food is an important consideration in such research because it may be an important factor in determining the acceptance of foods by fish (Liang *et al.*, 2001; Andrew *et al.*, 2004).

Moist diets are favoured over dry pellets by some fish species, particularly carnivorous species, which have been difficult to wean (Anderson, 1974; Liang *et al.*, 2001; Glencross *et al.*, 2002). Moist diets were found to be more palatable than dry diets in a number of species, including yellowtail, smallmouth bass, and tuna (Anderson, 1974; Cuzon *et al.*, 1975; Flickinger *et al.*, 1975; Glencross *et al.*, 2002). Moist diets may also be ingested more completely than dry pellets (Andrew *et al.*, 2004) and, when compared to equivalent dry diets, produce equivalent or superior growth and survival in juvenile fish (Cuzon *et al.*, 1975; Flickinger *et al.*, 1975; Bromley and Smart, 1981; Willis and Flickinger, 1981; Efthimiou *et al.*, 1994; Efthimiou, 1996).

Attempts to develop moist diets for species which refuse to accept dry diets have been made to address issues of cost, biosecurity, nutrition and feed use efficiency in the tuna industry in South Australia (Glencross *et al.*, 1999). Uptake of these moist diets

has been poor due to difficulties in converting fish from fresh diets to the moist pellets (Glencross *et al.*, 1999). Also, difficulties in transport and storage of some moist feeds may make them less attractive for large-scale commercial use (Glencross *et al.*, 1999). However, some moist diets have improved stability in water and may be more attractive than dry diets to some species, which is important for benthic feeding crustacea and fish (Flores *et al.*, 1994; Marsden *et al.*, 1997). The moisture content of moist diets (30-50% moisture vs <10% in dry diets) has not influenced growth rates in trials comparing isocaloric diets (Bromley and Smart, 1981; Otteraa *et al.*, 1994; Efthimiou, 1996; Moura *et al.*, 2000).

The aims of Experiment 4.2 were to determine whether moist diets were comparable to dry diets in terms of growth and survival of golden perch fingerlings, and whether feeding responses differed to these diets. If a moist diet proves to be more palatable than a dry pellet, and produces equivalent growth, it could be a promising option for aquaculture of a species which easily reverts to natural food types when introduced into a pond situation where natural foods are available.

4.2 Materials and Methods

4.2.1 Experiment 4.1. Effect of density on growth

Golden perch fingerlings obtained from a commercial hatchery were weaned in 2000 L tanks using methods detailed in Chapter 2, Section 2.2.1. Two weeks after weaning was complete, the fingerlings were coarsely graded, to remove extremes of large and small fish. A subsample of the coarsely graded fish had mean (±SE) SL of 34.65 mm ± 0.755 and weight of 1.132 g ± 0.078, (n=50). Fingerlings were then distributed into randomly assigned 100 L tanks at densities of 100, 200, 500 and 750 per tank, with four replicates for each density. Density was therefore 1000, 2000, 7500 and 10000/m^3, or 400, 800, 2000 or 3000 /m^2 (Chapter 2, section 2.2.1.2 for tank details). Biomass was 1.132kg/m^3; 2.264 kg/m^3; 8.49kg/m^3 and 11.32kg/m^3 respectively. Mortalities due to handling and grading were replaced from graded fish of the same cohort for the first ten days of the experiment. The water source for this experiment was irrigation channel water, filtered to 10 μm. Fish in each tank were fed to excess

(judged by the amount of uneaten food on the tank bottom after one hour) with 1 mm crumble salmon diet (Pivot. Co, Rosny Park, Tasmania). Proximal analysis of this diet is presented in Table 4.2. Crumble food was distributed evenly across the surface of the water in each tank in two rounds per feeding session, three times per day (at 08:00, 12:00 and 16:00h). Fish were closely observed, especially at feeding times and afterwards, and also while cleaning, to establish levels of aggressive interactions between fish. The experiment ran for 82 days, including the initial ten day period when mortalities were replaced. SGR was expressed as percentage growth per day (Busacker *et al.*, 1990)

Water temperature in the culture tanks ranged from 22°C to 29°C (mean 24.9°C), pH was 7-7.1, and oxygen was above 6 mg/L at all times. Periodic 24 hour oxygen profiles were done to check for diel variations in oxygen levels. Ammonia levels in two replicates of each treatment, plus inflow water, were measured weekly prior to cleaning tanks in the morning.

A sample of 50 fingerlings from each tank was measured (SL) and weighed (after blotting) prior to the start of the experiment. At the end of the experiment, a subsample of fifty fish was measured and weighed, and an additional subsample of fifty fish weighed only, from each tank. At weighing, fish were assigned a subjective condition factor based on whether the abdomen was sunken (non-feeding) or distended (feeding). A numerical condition index was also calculated using the equation $K = wt/SL^3$ (Busacker *et al.*, 1990) where weight is in grams and length in mm. The scaling constant of 10,000 was applied in this case (Rowland *et al.*, 2004).

Regression analysis was used as this experiment compared different densities, to determine if there were significant trends in weight, condition or number of 'feeding fish' between treatments. Linear and quadratic equations were fitted to the data to determine if there were significant trends in the treatments and also to reflect the spread of replicates. Plots and fitted equations were calculated for condition, weight, standard length and for the number of fish over 20 g in each sample of fifty fish (or of 100 fish for weight) to examine patterns of distribution and trends at changing densities. Means of replicates were not plotted and fitted because these would give only four points and a much less realistic impression of what really happened.

Regression of coefficient of variation data was not transformed, as transformation would have hidden the effect of the 'tail' and any differences at that end of the distribution. However, because the data were not normally distributed use of statistics like CV on non-transformed data need to be treated with caution.

The size frequency distributions of the different densities were compared using Kolmogorov-Smirnov test for differences between the distributions. Due to the large number of measurements (>400) in each sample the Kolmogorov-Smirnov test will find differences in cumulative distributions that are better demonstrated by examining histograms directly.

Experiment 4.1 was approved by the Far North Queensland Animal Ethics Committee, approval number FNQ- 01-01.

4.2.2 Experiment 4.2. Growth of golden perch fingerlings fed moist and dry formulated diets

Fingerling golden perch obtained from a commercial hatchery were air freighted to FFAC, given prophylactic formalin and salt bath treatments, and weaned in 2000L tanks as per the zooplankton method in Chapter 2. Two weeks after weaning the fish were graded (SL 38.50 ± 0.351mm, Wt 1.33 ± 0.032), and 100 were placed into each of 18, 100 L tanks see section 2.2.1.2 for details).

Three commercially available Australian manufactured crumble diets were used in the experiment. These were Kinta (K) (Yarrawonga, Vic.), Ridley's (R) (Narangba, Qld.) and Pivot (P) (Rosny Park, Tas.). Moist diets were formulated by grinding the crumble diet in a hammer mill then mixing it with 3% w/w gelatine, and extruding, drying, crushing and sieving to make a crumble similar in size to the dry formulated control diets. Prior to feeding, 30 mL of water was sprayed over 70 g of diet, which resulted in the diet reaching a soft to touch consistency but did not dissolve or glue together. The control dry diets were oven dried for the same length of time as the dry diets in an attempt to ensure that they had undergone essentially the same temperature treatment. Nutrient analysis of each diet is provided in Table 4·2. Diets were analysed

by the Animal Research Institute, Yeerongpilly, Brisbane. All methods used were standard methods. Vitamin A was measured as retinol, and vitamin E as tocopherol. Peroxide value is a measure of rancidity; the higher the value to more likely it is that fat could be considered rancid. It is an indicator of rancidity, not an absolute measure. Temperatures ranged from 24.02 to 26.7 °C (mean 25.8°C).

Feeding in all tanks was to excess, three times per day. Previous studies (Chapters 2 and 3) demonstrated that golden perch feed off the bottom of a tank for some time after food is offered. Tanks were checked for excess food on the tank bottom about 30 minutes after feeding. Fish were fed in two rounds as they were found to feed more avidly if fed after a first round. The experiment ran for 98 days. All fish from all tanks were weighed on days 58, 69 and 98, following heavy anaesthesia using Aqui-S (isoeuganol), and blotting on a moist synthetic sponge.

Due to the experimental design a split plot ANOVA was done on final weight data and on arcsine transformed survival and starvation data. The split was based on diet formulation (dry or moist). Weight data for each measuring period was tested; survival data was only tested for the end period. Genstat 6.1 was used for these analyses.

Experiment 4.2 was approved by the Far North Queensland Animal Ethics Committee, approval number FNQ- 08-01.

Table 4·2. Proximate analysis and indicator of rancidity (peroxide value) in moist and dry diets. Dry diets fed to fish were oven treated. All measures are on a dry matter basis. K = Kinta, R = Ridley's and P = Pivot.

Diet	% dry matter	% Ash	% Protein	% Lipid	% Crude fibre	Vit A mg/kg	Vit E mg/kg	Peroxide value
K dry	90	10.9	54.9	7.6	2.5	1.2	25	77.2
K moist	90.2	10.4	56.0	7.0	2.7	12.9	20	43.6
P dry	93.2	9.6	55.9	17.3	1.2	6.3	202	5.5
P moist	91.2	9.1	56.8	17.5	0.8	5.2	177	5.7
R dry	94.3	9.6	60.1	8.3	1.3	13.6	117	6.5
R moist	90.4	9.0	60.7	9.2	1.0	18.7	116	8.3

4.3 Results

4.3.1 Experiment 4.1. Effect of density on growth

4.3.1.1 Behaviour

In the two higher density treatments the fish stayed in a reasonably stationary tight mass near the bottom of the tank, except when feeding when they rose as a school and spread out at the surface to eat the crumble food. The fish in the two lower densities were more evenly distributed throughout the entire tank, from the top to the bottom. All fish in all treatments rose to the surface for feeding. No aggressive interactions were observed at any time.

4.3.1.2 Growth

The regression analyses present a trend towards reduced growth at higher density, although the high variation between treatments at the two higher densities reduces the R^2 value considerably (Table 4·3, Figure 4·1). SGR was lowest in the highest density treatment and relatively similar in the other treatments (Table 4·4). The weight of non-feeding fish was found to be always less than 20g, and the proportion of these non-feeding fish decreased as density increased (Figure 4.1). Condition factors increased marginally with density but were not significantly different. The size frequency distribution (Figure 4·2) indicates a similar distribution pattern across all treatments, although the highest density has the highest proportion of small fish at the end of the experiment. The two lower densities have approximately equal proportions of fish in each size class.

Table 4·3 Regression tests for density effects in golden perch density trials from the final measurements.

Graph	Test of significance	R^2
SL	<0.01	65.3%
Weight (n=50)	<0.01	65.7%
% of fish >20 g	<0.01	47.1%
No. of fish >20 g	0.015	35.5%
Condition	0.074 (not significant)	21.0%

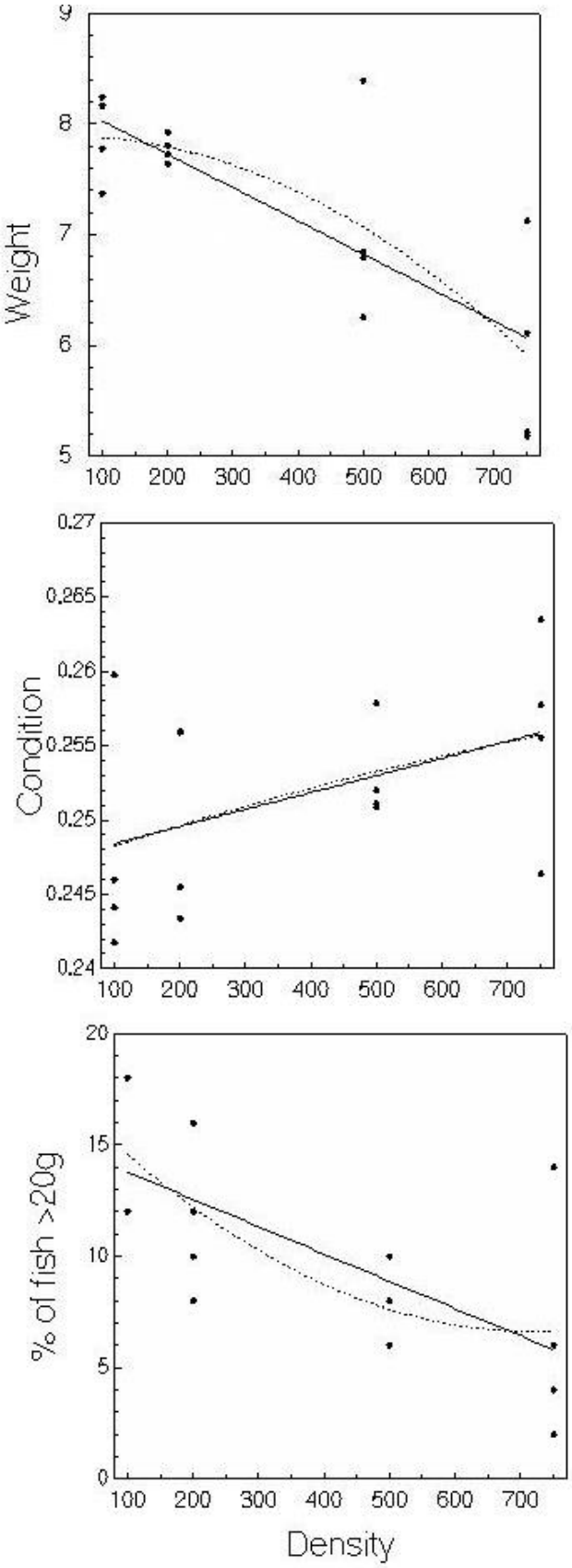

Figure 4·1 Regressions for density against mean weight, condition and percentage of 'feeding fish' for each replicate treatment. Each data point represents the values of a single replicate for fish measured on day 52.

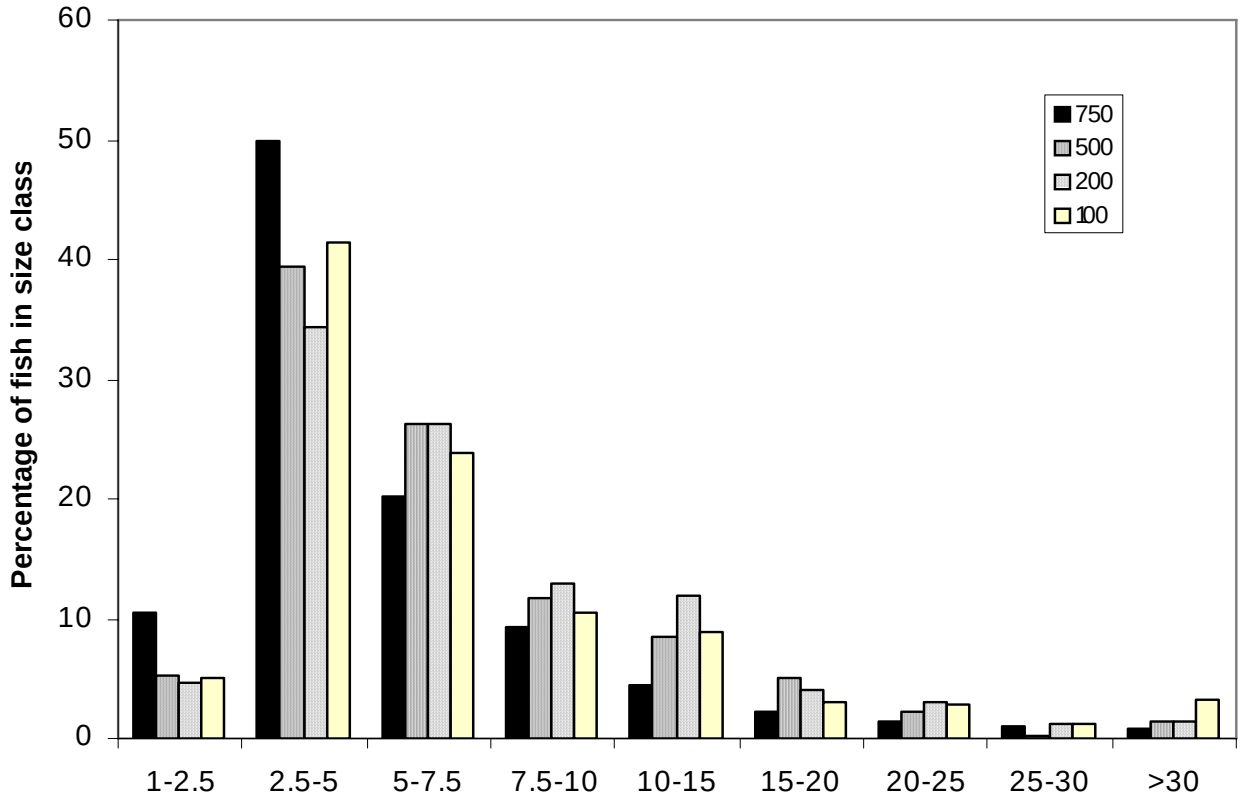

Figure 4·2. Size frequency distributions of golden perch fingerlings cultured for 82 days at four different densities (750, 500, 200 and 100 per 100 L tank).

SGR value was significantly lower for fish grown at a density of 750 fish per tank than all other density treatments (Table 4·4). The coefficient of variation was highest in the lowest density fish population and between 75-80% in the three higher density treatments (Table 4·4).

Table 4·4. Mean (± SE) weight, CV of weight, survival, condition and SGR of juvenile golden perch cultured for 82 days at four different densities. The same superscript indicates no significant difference (P<0.05).

Density (fish/ 100 L)	Mean weight (g)	CV weight (%)	Survival (%)	Condition	SGR
750	5.909 ± 0.27^{a}	75.2	99.82 ± 0.176^{a}	0.256 ± 0.004^{a}	0.0194
500	7.071 ± 0.27^{b}	70.9	99.81 ± 0.174^{a}	0.253 ± 0.0034^{a}	0.0230
200	7.776 ± 0.23^{b}	77.4	99.81 ± 0.189^{a}	0.250 ± 0.0034^{a}	0.0236
100	7.894 ± 0.39^{b}	99.5	99.83 ± 0.187^{a}	0.248 ± 0.004^{a}	0.0252

Fish in the high density treatment showed a significant difference in growth from fish held at other densities (Table 4·3). The next highest density (500) had lower weight gain than the two lowest densities, being 0.705 g lighter than the 200 fish treatment. Ammonia levels never exceeded 0.007 mg/L unionised ammonia, and water temperature ranged from 22.1° to 29°C, with diurnal variation of about 4°C. Oxygen levels in all treatments always exceeded 6mg/L.

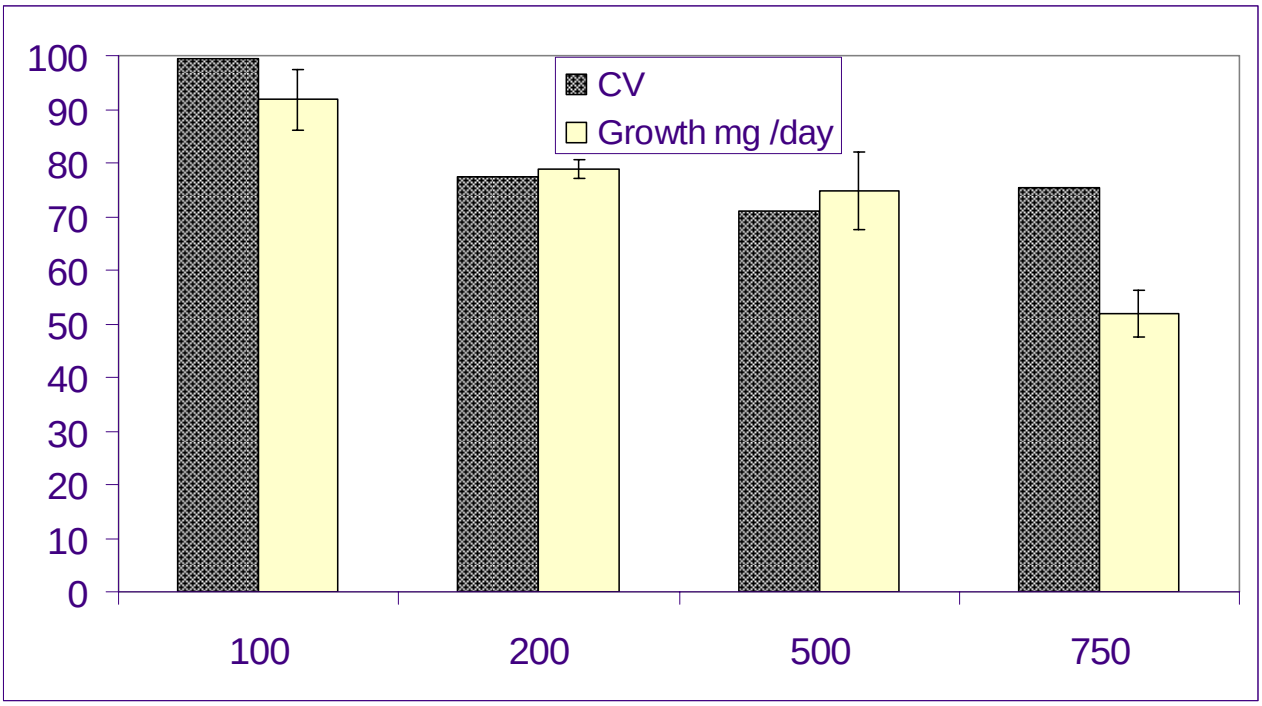

Figure 4.3. Coefficient of variation (%) and growth rate (mg/day) of golden perch cultured for 82 days at four different densities. Error bars represent SE between replicates.

4.3.2 Experiment 4.2. Growth of golden perch fingerlings fed moist and dry formulated diets.

There were no substantial differences in the percentage dry matter, protein content or carbohydrate levels of the diets (Table 4·2). Diets K and R had substantially lower levels of fat than diet P (a salmonid starter diet). Diet K also had much lower levels of vitamins A and E and higher peroxide values and crude fibre than the other two diets. After addition of 30% by weight of water these levels would have been reduced by

30%. The addition of gelatine to the diet increased the amount of vitamin A in the diet, and generally only produced a marginal increase in protein level.

Golden perch fingerlings fed on two of the dry diets performed significantly better than the moist diets, but those fed diet K performed poorly whether dry or moist. The growth rates of fish fed the moist diets and dry diets are presented in Figure 4·4. Fish fed on the dry diets R and P gained weight significantly better than those fed on the other diets. Fish fed on dry diet P performed better than the others in terms of growth over the entire length of the experiment (Table 4·5).

Table 4·5. Mean (± SE) weights of golden perch throughout the experimental period. The same superscripts indicate no significant difference (P<0.001, except at day 98 P<0.007) between diet formulations (K,P,R) or texture (moist/dry) and interactions.

Formulation	Days from start	K	P	R
Dry	54	1.670 ± 0.020 [a]	2.352 ± 0.043 [b]	2.136 ± 0.043 [c]
Moist		1.651 ± 0.028 [a]	1.602 ± 0.032 [a]	1.757 ± 0.031 [a]
Dry	69	1.739 ±0.023 [a]	2.741 ± 0.054 [b]	2.380 ± 0.05 [c]
Moist		1.869 0.034 [a]	1.758 ± 0.04 [a]	1.879 ± 0.043 [a]
Dry	98	2.079 ±0.038 [c]	3.827 ± 0.095 [a]	3.369 ± 0.093 [a]
Moist		2.310 ± 0.054 [c]	2.443 ± 0.069 [c]	2.596 ± 0.071 [b]
SGR				
Dry		0.60	1.29	1.15
Moist		0.75	0.78	0.84

At the end of the experiment 15 fish of each treatment (five from each tank) were euthanized and the stomach examined after feeding, In all cases the stomach was grossly distended with crumble food particles. All fish appeared to have eaten to satiation, that being the physical limitation of the stomach to hold food. There was no apparent difference in food consumption rates on a gross level.

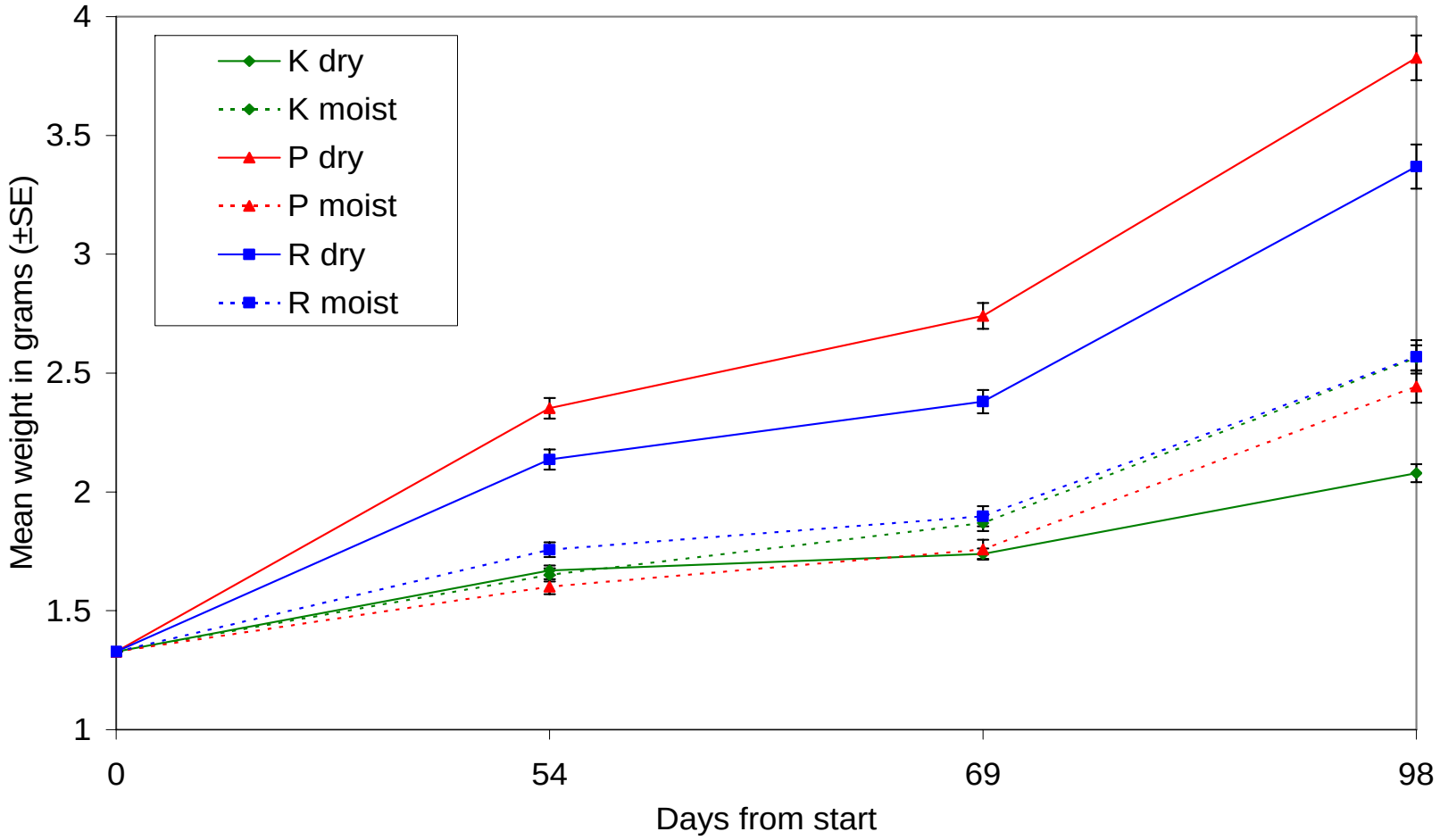

Figure 4·4. Mean (± SE) weights of fish fed dry diets (solid lines) or moist diets (broken lines).

Survival of fish fed moist or dry treatments was not significantly different at the 5% level, except for the moist P, which had significantly lower survival of 84.7% (Table 4·6). There was no significant difference in number of starved fish in any treatments at the 5% level.

Table 4·6 Survival of fish fed moist and dry diets after 98 days. The same superscripts indicate no significant difference in survival between treatments.

Diet	K	P	R
Dry	98.5 [a]	99.6 [a]	98.1 [a]
Moist	95.5 [a]	84.7 [b]	92.0 [a]

4.4 Discussion

4.4.1 Experiment 4.1. Effect of density on growth

The tight schooling effect of the fish in the high density treatment suggests that at high density, the fish school; whereas they do not in lower densities. It may be that in the tank, the darker colour of the school attracts the fish to it. Schooling did not occur in the lower density treatments where fish were evenly distributed throughout the water column. This could have important implications in aquaculture as, in preliminary studies, development of hierarchical dominance was observed in golden perch (Stevenson and Grant, 1957). Condition factor increased with increasing density and there was less variation in final size in the highest density.

Growth heterogeneity in Eurasian perch is largely determined by social interaction, probably due to limited access to food at lower density (Mélard *et al.*, 1996a). A similar situation appears to exist with juvenile golden perch in tanks, as size frequency distribution showed a greater number of large, and possibly dominant individuals, in low density treatments. In golden perch culture the trade off between higher overall production at higher density, and size variation at lower density, will need to be considered.

The differences in weight gain reflect a split between the two higher densities and the two lower densities. Growth was reduced at the higher densities, suggesting that in this situation a stocking rate of somewhere between 200 and 500 fish per 100 L tank is the maximum density that can be attained with minimal effect on growth. Many other species of fish have suppressed growth rates at high densities for which the reasons are behavioural (Baras *et al.*, 1998; Maragoudaki *et al.*, 1999; Anderson *et al.*, 2002; Jodun *et al.*, 2002; Saillant *et al.*, 2003). The growth of golden perch at the lowest density was significantly different to those in others, suggesting that low density culture, under the conditions trialed, will be best for maximum growth in this species. As noted above, there did appear to be some behavioural influence on growth, which would have to be balanced against increased overall growth. It is possible that the feeding frequency may have restricted growth to a degree.

The regression figures demonstrate a trend to reduced growth with increasing stocking density, suggesting that golden perch is one species in which density influences growth rates. This has been shown for with many other fish species where increasing density has a negative effect on growth (Henderson-Arzapalo and Stickney, 1982; Oellermann and Hecht, 1998; Chude, 2001; Gornati *et al.*, 2004). However, the wide spread of data points in high density replicates suggests that they were more variable than the low density treatments. The regression of CV does not bear this out as all replicates of treatments had high CV and also a large spread of CV. This indicates an intrinsic high variability in growth rates which is not influenced by density. The reduction in percentages of large fish in the high density does suggest that there may be a suppression effect on growth of faster growing fish, which is not apparent in the lower densities. There was a slight trend for increased condition in the higher density, but again a low R^2 value and wide spread across replicates. The regression of the percentage of fish over 20 g suggested a decline in the number of larger fish at higher density, but again there was a wide spread between replicates. Although the test for significance indicated it was highly significant, the low R^2 value (0.471) and spread of the points suggest that further clarification is needed.

Ammonia levels in tanks never exceeded 0.0073 mg/L, which is well below levels that impact on fish growth (Frances *et al.*, 2000). At the densities and flow rates in this experiment, build up of toxic metabolites did not affect fish health.

The results of this study indicate that juvenile golden perch (55-65 mm SL, 7-8 g) will grow well at a density of up to 2-3 kg/m^3 in a tank system. However, increased feeding periodicity could change this, to allow stocking at higher rates than suggested by this trial.

4.4.2 Experiment 4.2. Growth of golden perch fingerlings fed moist and dry formulated diets.

Growth of juvenile golden perch on two diets was depressed when those diets were modified to be moist, soft texture diets. This is in direct contrast to the results of most studies where moist diets were superior to dry diets. In a study on cod (*Gadus morhua*), SGR increased with increasing dietary water content (Otteraa *et al.*, 1994). Cod is a marine species which may benefit physiologically from having extra water included in the diet. Another marine species, common dentex (*Dentex dentex*), also grew faster and had better food conversion ratio (FCR) on moist diets than dry diets (Efthimiou *et al.*, 1994). However, the poor performance of common dentex on dry diets was not explained by the different moisture content (Efthimiou, 1996). In trout, a freshwater fish, increased moisture levels in the food had no benefit in terms of growth, when dry matter intake was equal for fish fed either dry or moist diets (Bromley and Smart, 1981). Dry diets were found to give better FCRs and less wastage than moist diets in largemouth bass, due to the moisture content in the food (Kubitza and Lovshin, 1999).

The most likely reason for the poorer performance of golden perch fed moist diets is the quantities of food ingested. The fish avidly consumed all the diets until satiation. Examination of stomachs of the fish, and the gross distension of the abdomen suggested that they ate until it was physically difficult to ingest more food. As the moist diets in this experiment were 30% water, they would have had comparatively less dry matter intake than fish which had eaten the dry diet. The potentially long gut retention time of food (96 hours)(Anderson and Braley, 1993) could allow higher food efficiencies if feeding régimes were altered to every second or third day instead of daily.

Fish appeared to feed marginally more avidly on the moist diet than dry diets, although, as stated above, all fish appeared to eat to satiation. Moist diets have been found to be more palatable than dry diets in the few studies where direct comparison is possible (Anderson, 1974; Cuzon *et al.*, 1975; Flickinger *et al.*, 1975). Many of the moist diets trialed have used fresh fish (i.e. Oregon type diet) so are not directly comparable as they may have more attractants than a dry diet made with fish meal. However, in a study on peacock bass (*Cichla* sp.) the attractants cod liver oil and krill meal were not found to be beneficial (Moura *et al.*, 2000).

Survival between treatments was not significantly different, indicating that the quality of food eaten was sufficient for fish to survive and grow, although those fed on diet K and gelatine treated diets grew much slower than those fed on dry diets P and R. Few other studies of moist and dry diets have reported the survival of fish as being different, except in common dentex (*Dentex dentex*) where agonistic behaviour was affected by food type offered (Efthimiou *et al.*, 1994).

It is unlikely that gelatine binding affects digestibility of the food, as gelatine has been found to have high digestibility in other fish species (Schmitz *et al.*, 1982; Grove *et al.*, 2001). Fish fed dry diet K performed poorly compared to those fed moist diet K, suggesting that addition of gelatine to the diet may have increased its nutritional value. This discounts gelatine as a possible agent in negative impacts on growth of fish fed on the other moist diets. The gelatine treated diet K had substantially lower peroxide values and higher vitamin A and E levels, which may have enhanced performance of fish fed on it compared to the comparatively rancid dry diet. Vitamins A and E have powerful antioxidant and immunostimulant properties in fish which can help to increase growth rates (Esteban *et al.*, 1999; Mourente *et al.*, 2002).

Although use of moist feeds may be of benefit in some species, in this trial the amount of water required to make a soft, moist pellet reduced the amount of dry matter ingested by the fish, resulting in reduced growth rate. The treatment of the diets did not appear to reduce the levels of vitamins or macronutrients where these were at relatively high levels prior to treatment. The gelatine appeared to have the effect of bulking up the food eaten, a technique used in human nutrition to reduce weight gain

(by use of mucilages and soluble fibre to induce a feeling of fullness). Possibly a similar phenomenon occurs in the fish as excretion of water is possible only after digestion of the diet commences.

The water content of the moist diet may have incurred a physiological energy cost by increasing energy needed to excrete water from the fish. The reduced growth is most likely due to the relative reduction in amount of dry matter consumed. In fish such as golden perch, where food consumption appears to be limited by stomach size, diluting the diet with water reduces growth substantially so it is not worth considering for juvenile fish in a tank situation.

4.5 Conclusion

Studies of fish behaviour and reaction to various experimental treatments in tanks can only be used as a guide for behaviour in larger tank systems or ponds. However, because of the numbers of fish involved, and intrinsic between pond variation, replicated trials by necessity must be conducted in small, easily managed units. The results of these trials demonstrated that high density in small volume negatively impacted growth, and that this was not due to poor water quality of the parameters measured. The 'space' issue (i.e. freedom to move around a larger area, even in higher density) can be a strong influence on growth rates. The results of Experiment 4.1 give guidelines as to numbers that could be stocked in weaning tanks or nursery raceways with little impact on growth. Around 200 fish per 100 L (i.e. 2000/m^3) could be stocked without negative impact on growth according to the results of these studies. Possibly in larger size tanks even more fish could be stocked due to schooling behaviour and larger area available.

The unequivocal results for Experiment 4.2 demonstrate a clear need for a concentrated diet for this species of fish. Previous experiments which have proven moist diets better than dry diets were largely due to lack of ingestion of dry diets by the experimental subjects. The apparent limitation of golden perch to ingest food (by the size of the stomach) suggests that highly concentrated food would be preferable to less nutrient dense food. Higher food efficiencies are required as golden perch only

require 40% dietary protein level for optimal growth and higher levels do not improve growth (McFayden, 2001). Thus, energy rich diets, approximating protein:lipid ratios found in the invertebrate foods of wild golden perch may be the appropriate feed. Moist pellets may, however, have a place if they improve food stability in the water and thus decrease leaching of nutrients or loss of food during processing by the animal (Marsden *et al.*, 1997). However, increased processing and storage of a perishable moist product do limit its potential applications.

These experiments demonstrated that some tank experiments have a place in determining experimental protocols to assess conditions for pond culture, and do assist in defining parameters for tank based systems. The replication levels possible and absence of differences in environmental factors permit accurate testing of particular parameters such as diet, weaning techniques and handling in tank systems. However, the real task of applicability is in the approximation of the pond based system, experimental ponds, where abiotic factors (e.g. weather) and biotic factors such as (e.g. natural productivity, alternative food sources) add an element of challenge in interpreting growth data.

Chapter 5
Pond Nursery Trials-Effects of Density and Feeding

5.1 General introduction

Populations of golden perch stocked into impoundments or rivers indicate that they can grow to between 285 and 835 g in two years (Cadwallader and Backhouse, 1983; Barlow *et al.*, 1987; Battaglene, 1991; Sissins, 2004). The preferred market size of golden perch is 400-700 g (Ruello, 2000), and the market accepts fish as small as 250 g (Graham, 2004a) indicating that golden perch growth rates are sufficient to produce marketable fish in less than two years. This, combined with the reduced catches, augers well for development of aquaculture of the species in Australia. Millions of fingerlings are produced in hatcheries for recreational fishery enhancement (Diver, 2000; Hamlyn and Cheetham, 2001; Watson, 2001), and this ample supply of fingerlings is increasingly being sold for aquaculture (O'Sullivan and Savage, 2004).

Although some studies have documented growth rate of hatchery reared golden perch stocked into natural waterbodies (Barlow *et al.*, 1987; Sissins, 2004), only one study has been published on golden perch stocked into ponds and fed (Stevenson and Grant, 1957). That study had difficultly in finding a suitable food source and obtaining measurements of growth rate, but did report dominance behaviour, effect of disturbance on feeding, and surprisingly high but variable food consumption (6-7% wet weight of fresh mollusc per day) that could not be explained by external factors. Food consumption decreased at temperatures below 24 °C (Stevenson and Grant, 1957). Golden perch are benthic feeders and are opportunistic macrophagic carnivores (Barlow *et al.*, 1987; Battaglene, 1991), so potentially may be amenable to taking formulated feeds.

Golden perch are widespread throughout inland Australia, which is prone to extended periods of poor water quality and inadequate food resources. When food resources become available they gain weight and condition rapidly (Collins, 1996). Rapid growth potential is one prerequisite for aquaculture.

While the hatchery production of golden perch fingerlings, which was developed in the 1970's and 1980's, is well established (Rowland *et al.*, 1983; Rowland, 1984; 1996), growout of golden perch for human consumption has only recently been investigated. The major hurdle to development of an industry has been the perception that golden perch will not readily accept pellet foods (Anderson, 1986; Fallu and Mosig, 1994). However, the development of a reliable and successful weaning process (Chapter 2) overcame this constraint, to the extent that in 2003/4, 1.8t of golden perch was produced from aquaculture whereas previously there had been no aquaculture-produced golden perch. Some commercial farms have weaned golden perch with up to 80% success, but still rely largely on natural production in pond grow out (Mosig, 2001).

This Chapter reports on three experiments which developed methods for pond culture of golden perch and examined problems or issues relevant to growout in a commercial situation. Specifically, feeding in ponds was studied as a possible reason for the uneven growth, and attempts made to identify the causes for it.

To address the lack of data on potential growth rates under aquaculture conditions, Experiment 5.1 was conducted using weaned fingerlings. Data on growth rate under aquaculture conditions, effects of density on growth rate and size frequency distribution, problems with disease or other management issues, food conversion ratios and specific growth rates were collected to determine whether golden perch will adapt well to aquaculture conditions.

Techniques have been developed for pond aquaculture of silver perch, which are sympatric with golden perch. There have been several studies on culture of silver perch at two different densities, in order to establish whether they could be grown at commercial densities (Table 1)(Rowland, 1994; Rowland *et al.*, 1994; Rowland, 1995b; Rowland *et al.*, 1995; Harpaz *et al.*, 2001; Rowland *et al.*, 2004). These silver perch experiments were used as a model to design Experiment 5.1.

The aims of Experiment 5.1 were to determine if golden perch could be grown in high density pond culture, observe what growth rates were, and determine what pond management techniques were required. Additionally, issues with growth

heterogeneity, hierarchy development or other behavioural attributes which would require management needed identification.

Table 5·1. Experimental work on silver perch at different densities. Most experiments indicated small differences in growth between the densities trialed.

Density	System	Start size	Final size	Duration	Source
25000 and 80000/ha	Ponds	0.6 g	16.0 g 7.4 g	12 weeks	(Rowland *et al.*, 1994)
7000/ha 21000/ha	Ponds	15.3 g	473.2 g 434.9 g	10 months	(Rowland *et al.*, 1995)
43000/ha	Ponds	4.6 g	402.4 g	14 months	(Rowland, 1995b)
50/m^3 100/m^3 200/m^3	Cage	2.3 g	112.6 g 122.0 g 114.3 g	20 weeks	(Rowland *et al.*, 2004)
50/m^3 100/m^3 200/m^3	Tank	2.3 g	50 g 48.4 g 41.3 g	20 weeks	(Rowland *et al.*, 2004)
30/m^3 60/m^3 90/m^3 120/m^3	Tank	50 g	≈100g ≈100g ≈100g 109g	10 weeks	(Harpaz *et al.*, 2001)

The results in Experiment 5.1 were confounded by lack of replicates and variability in growth rates in the two low density treatments. A large tail of slow growing golden perch was identified in Experiment 5.1, and the reasons for this had to be ascertained. Generally, either nutritional factors or behavioural factors are responsible for reduced growth. In silver perch, natural food growing in the pond or in cages is suspected to contribute to higher growth rates than those observed in tanks (Rowland *et al.*, 2004), although this has not been confirmed by gut content analysis. The number of

replicates of both densities also had to be increased to determine which mean growth rate in low density treatments was normal, and if there was a repeatable significant difference in growth rates between low and high density treatments of golden perch grown out in aquaculture.

Experiment 5.2 was designed to determine why some fish grew faster than others in the aquaculture situation, in two densities. Gut contents of the fish in nursery ponds were sampled to determine:

- the effect of density on diet composition,
- the proportion of fish that reverted from formulated feeds back to natural food sources after introduction into ponds, and
- the growth rate of these fish compared to those that ate mostly formulated food.

One issue with golden perch aquaculture development, identified in Experiment 5.2, was the retention of weaned fish on feeding on formulated foods after introduction into ponds. Experiment 5.2 indicated that the fish which reverted back to feeding on natural food in the pond did not grow, and is was suggested that broadcast feeding might ameliorate this situation. Point feeding is implicated as on contributor to runting in silver perch grown in commercial operations in New South Wales (Rowland and Walker, 1995). Comparing size frequency distributions and gut contents of broadcast and point fed golden perch may provide answers to whether behavioural characteristics, which may influence access to food, are important in retention on formulated diets.

After Experiment 5.2, fish populations were devastated by an infection with *Tetrahymena*, a hymenostome ciliate parasite of freshwater fishes. This parasite is believed to be favoured by high organic loads in the water (Callinan and Rowland, 1994), and with this in mind methods to improve water quality and reduce organic loads were investigated. Use of probiotics was one potential method of improving water quality which was practical and appeared to be economically possible. Probiotics are benthic bacteria used extensively in Penaeid prawn aquaculture to reduce viral and *Vibrio* related disease losses (Devaraja *et al.*, 2002). The process works by producing and maintaining high concentrations of beneficial bacteria in aquaculture ponds. These bacteria then compete with or inhibit undesirable bacteria

(Chandrika, 1996; Jory, 1998; Moriarty, 1998), and appear to also have a negative effect on virus transmission (Gatesoupe, 1999). They are also claimed to assist in breaking down organic matter and nitrogenous waste in the pond. Some studies have found reduced mortality from disease and greater net production from treated ponds (Verschuere *et al.*, 2000; Devaraja *et al.*, 2002). However, most studies have not detected any effect partly due to the variability of parameters in aquaculture operations and subsequent difficulties in quantifying positive results (Queiroz and Boyd, 1998; Gomez-Gil *et al.*, 2000; Shariff *et al.*, 2001). Due to the losses of golden perch to mixed motile Aeromonad/*Tetrahymena* infections, probiotic bacteria were trialed as a component of Experiment 5.3 to determine whether a commercially available product had an effect on health or water quality in nursery phase of golden perch culture.

The aims of Experiment 5.3 were to determine:

- whether broadcast feeding has any effect on size frequency distribution and ultimately weight gain at the end of the nursery phase;
- proportion of pellet feeding fish; and
- sizes of fish eating pellets as opposed to those which had reverted back to natural foods.

An additional aim was to trial a commercially available probiotic (used in commercial aquaculture) to assess whether its use in a freshwater aquaculture situation would affect water quality or disease susceptibility of golden perch in this experimental system.

5.2 Materials and Methods.

5.2.1 Pond and cage facilities

Trials were conducted in ponds of approximately 320 m^2, with a maximum depth of 1.2 m and a volume of 230 m^3. They were polyethylene lined, with a 300 mm deep layer of topsoil over the flat bottom part of the pond (20% of surface area). A 25 mm diameter polyethylene pipe with holes drilled into it was placed in the centre harvest trench for aeration, except in the first trial where airlifts were used initially, then

aspirators. Aeration was supplemented with aspirators when morning oxygen levels decreased to below 5 mg/L dissolved oxygen. Aspirators had fish fingers installed to prevent contact of fish with the impeller.

Prior to releasing fish into the ponds, fish were held in a cage located in each pond. Cages (1.6 m x 0.8 m x 1.2 m deep) were made of 3 mm semi-rigid plastic mesh supported in a PVC pipe float with a 90% shade cloth cover over the top, that extended down one side of the cage to 1 m depth. An airstone was provided to each cage. Cages were used to confine fish for observation to ensure they were eating artificial food after transfer into the pond environment.

5.2.2 Data collection techniques

Sampling started one hour after the morning feed, and was complete thirty minutes later. Ponds were sampled on each of three consecutive days to minimise time differences in sampling. Ponds were drained by about 30%, and a seine net pulled over about half of the pond. The partly drained ponds were refilled on the same day. A random sample of 51-53 fish was collected from the seine net, and transported to the hatchery in 0.02 mL/L Aqui-S solution in water. They were then measured and weighed using digital callipers and balance. Fifty fish were measured each time, with a few extras sampled in case of deformed or unusual fish in the sample. There was a small degree of spinal curvature in all batches of fish (<1%). Data from these samplings was used for plotting average growth rates and size frequency distributions.

When fish in a cage were fed, the cover was partly pulled back, the air stone removed, and the food sprinkled onto the surface. Fish were fed three times per day, at about 08:00, 12:00 and 16:00h. After release of fish from the cages into the pond, a 1 m diameter feeding tray adjacent to the shelter was checked one to two hours after each feed, and amount of feed offered adjusted accordingly. Food sizes on offer were mixed to allow for the range of gape sizes of fish based on monthly measurements. Feed sized varied from 1mm crumble through to 8mm pellet over the course of the experiment, mixture of pellet sizes were determine from observations of uneaten feed on food trays. Sinking pellet diets were used in all trials, as it had been found in preliminary trials that golden perch would rarely feed at the surface in ponds. Food

was measured volumetrically, and weights calculated from volume of food fed. As feed rates were continually being adjusted there was considerable overfeeding.

Pond preparation consisted of drying for several months, spreading with 10 t/ha calcium carbonate (agricultural lime) and then filling with irrigation water from a nearby reservoir. This water was filtered using a 500 μm plankton net sock to prevent entry of aquatic plants, fish and crustaceans. Fish were usually stocked immediately into the pond the day after filling, to minimise amount of plankton in the water.

5.2.3 Measurements and handling of fish

Fish were transported to the ponds in water with 10 g/L NaCl and 0.02 mL/L Aqui-S, and acclimated by gradual mixing of pond and tank water for about 15 minutes before release into the cage.

Fish to be measured and weighed were heavily sedated using 12 mg/L of Aqui-S, then laid on a damp synthetic sponge and length measured using callipers. Standard length (Strauss and Bond, 1990) was the measurement taken. Fish were then weighed, and returned to a bin of fresh pond water treated with 10‰ salt with 0.02 mL/L Aqui-S, for at least one hour prior to return to the pond. Pond water was gradually mixed with bin water for 15-20 minutes before the fish were released back into the pond of origin. During measuring, any unusual characters (marks, abrasions, lesions) were noted and general health of fish assessed. All measurements reported are means ± standard error. Callipers and balance were linked to a computer for download of data via an RS232 port, and were calibrated annually.

A random sample of twenty fish was collected from each pond using the above techniques every two weeks for gut analysis in the second and third nursery trials. These fish were weighed, then overdosed with Aqui-S gradually (to prevent regurgitation of food), and frozen for later examination. Frozen fish were defrosted, weighed, and the entire gut removed. The stomach and intestine were cut apart at the duodenum, and contents of the stomach and intestine examined separately. Insects were identified to family level, genus where possible and development stage (nymph, pupa etc.), and crustaceans to genus. The relative proportion of each food type was

estimated and recorded as a percentage. If the stomach or intestine was not full, it was noted as either half full or empty.

5.2.4 Water Quality

Water quality (pH, temperature, DO) was measured at least twice weekly with regular checks of morning DO and afternoon pH, to ensure that morning DO was always >5 mg/L at 07:30 to 08:30h, and pH < 9 at 14:00-16:00h. Temperature was monitored hourly using calibrated temperature loggers. In Experiment 5.3 (probiotics/broadcast feeding), pH and oxygen were measured twice on every day of the trial, at about 08:00 and 1500h. Total ammonium-nitrogen (NH_4-N) was tested weekly in the three ponds that had the highest pH. If pH exceeded 9·0 or ammonium-nitrogen exceeded 0·8 mg/L, the pond level was dropped by about 70% and refilled. In Experiment 5.3 pH tolerance was raised to 9·5 as disturbance associated with more frequent water changes appeared to affect fish feeding.

Water quality was measured using a TPS 90FL meter with temperature, oxygen, pH and conductivity probes. Oxygen readings were salinity compensated. The meter was calibrated weekly. Oxygen membranes and electrolyte solutions were replaced as necessary, or once a month. Ammonia readings were taken using filtered pond water on a Palintest spectrophotometer which had been checked for accuracy against a Hach DR2000 spectrophotometer and by measuring ammonia standards of known concentration. Palintest or Hach test kits were also used to check hardness (calcium and total) and alkalinity periodically.

5.2.5 Experiment 5.1. First Pond Nursery and Growout Trial

Golden perch fingerlings (20.57 ± 2.0 mm and 0.2024 ± 0.0076 g) were received from a commercial hatchery and weaned in 2000L tanks onto a commercial Murray cod starter diet (Kinta Pty Ltd, Mulwala NSW. 50% protein, 8% fat, 2% fibre, 10% ash) using the method described in Chapter 2, Section 2.5. After 128 days they were transferred to four experimental aquaculture ponds. Aeration was supplied by airlifts at the beginning of the trial and supplemented by aspirators (after day 130 in ponds)

on timers providing aeration from 24:00 to 07:00h. The fish were transported to the ponds in 7‰ salt water, and acclimated by sitting the transport bin in the pond for 20 minutes, then gradual mixing of pond and tank water for about 15 minutes before release into cages. Fish were held for an acclimation period of 14 days in a cage located in each pond. There were two replicates (ponds) of each density (105,000 fish/ha or 31,250 fish/ha). Fish were stocked into ponds at 50.3 ± 1.35 mm and 3.55 ± 0.302 g.

After acclimation, fish were released by turning the cage onto its side allowing the fish to swim under the cover provided by the shade cloth attached to the float. Feeding behaviour in cages and after release was monitored. Feed was a mix of Kinta number 3 cod grower crumbles (up to 3 mm diameter, 52% protein, 10% lipid) and was mixed with Kinta 3 mm cod grower (52% protein, 7.7% lipid, 10% ash, 28% carbohydrate) as fish grew.

Fifty fish were sampled (weight, length) every four weeks after stocking, and one extra sampling done 14 days after release into the pond. Fish were not fed on the morning of sampling. Initially fish were fed three times per day, but the midday feed was discontinued on day 66, as food consumption was negligible.

FCR and SGR were calculated from monthly growth figures and food consumptions. FCR was calculated using the equation food weight fed/wet weight of fish; SGR by the equation:

$$SGR = \frac{\text{ln final weight - ln initial weight}}{\text{time in days}} \times 100$$

expressed as a percentage growth per day (Busacker *et al.*, 1990).

Condition factor was calculated as $K = wt/SL^3$ where weight is in grams and length in mm. A scaling constant of 10,000 was applied (Rowland *et al.*, 2004). Growth and condition data in October was analysed using ANOVA in Genstat 6.1. At harvest all fish were hand graded into three size classes, small (<90 g), medium (90-200 g) and large (>200 g) for further grow out. All fish harvested were counted while grading to determine survival rate.

Size frequency distributions were predicted from the second last measuring period and applied for grading at final harvest. Measurements from the final measuring event were used to produce size frequency histograms for each pond. Size frequency data for the two densities was compared using Kolmogarov-Smirnov two sample test to test for significant difference in the distributions of the two densities. For this analysis the data from pond B2 were used without transformation, and compared to the data from other ponds collected ten days later. The experiment ran for 221 days until a power failure resulted in death of most fish in one pond.
The experiment was approved by the Far North Queensland Animal Ethics Committee, approval number FNQ- 06-00.

5.2.6 Experiment 5.2. Nursery production of golden perch at two densities in ponds

Fourteen thousand fingerling golden perch (SL 27.6 ± 0.48 mm, Wt 0.43 ± 0.028 g, n=50) were air freighted from a commercial hatchery to the Freshwater Fisheries and Aquaculture Centre, Walkamin, Queensland. On arrival fingerlings were treated for ectoparasites with 10 g/L NaCl and 70 mg/L formalin for one hour. Twelve hours after arrival, they were again treated with 10 g/L NaCl. They were split up into six, 2000 L tanks in a subdued light environment (average light intensity between 08:00 and 18:00h was 1.96 lux, see section 3.2.3 for explanation of light readings). Thereafter, they were treated with 10g/L salt once a week to control ectoparasites. Frozen zooplankton blocks were fed during an acclimation period of ten days, then fish were weaned on to a formulated crumble diet (salmon starter 1mm crumble, Pivot Co, Rosny Park, Tasmania. 50% protein, 14% lipid, 9% ash) over a period of ten days (see Chapter 2). Five days after weaning was completed, fish were counted and graded, then held for a further five days before the trial commenced.

After nine days in a cage in each pond, fish were released by turning the cage onto its side, allowing the fish to swim to the cover provided by the shade cloth attached to the float. A sinking barramundi diet was used for the duration of the trial (Pivot Co, Rosny Park, Tasmania. 50% protein, 14% lipid, 9% ash). Feeding behaviour in cages and after release was monitored to ensure that weaned fish continued to feed on the

formulated diet. Feed was distributed in an approximately 3 m^2 area directly above the 0.6 m^2 feed tray adjacent to the shade cloth shelter.

A sample of fifty fish was measured every four weeks during the trial, commencing nine days after release into the pond. There were three replicates (ponds) of each density (95,300 fish/ha or 32,800 fish/ha; 3050 or 1050 fish per pond). Fish were sampled by flow trap in the first week, and by seine net thereafter. Samples of twenty fish were collected every two weeks for gut analysis. After 126 days, all fish in all ponds were harvested and a sample of fifty fish was randomly collected from each pond harvest, weighed and measured. One night sample was done one hour after the evening feed to determine if there were temporal differences in food composition in the gut. This was done in between the normal two weekly samples.

Analysis for difference in growth patterns between two densities was done using REML (repeated measures analysis), which fits a mixed model estimated by REML. This process can be thought of as an extended regression model, the extension required to handle the serial correlation by repeatedly sampling the same ponds. Intra-class correlation is also accounted for, due to the multiple measures for each pond at each time. Using a mixed model allows modelling of the response of weight dependent on time while simultaneously modelling the correlation structure. Splines were applied to help describe the relationship. Data was log transformed to reduce skew of residuals. To overcome heterogeneity in the residual variance, a separate error variance was fitted each time.

To answer whether there was variation within a pond for different densities, a mixed model estimated by REML was fitted where there was a fixed effect for each pond and the error variance was broken into two components (high density and low density). The variance components and their associated degrees of freedom were then compared using Bartlett's test for homogeneity. This was only done on data from the 30th April. Raw data for this analysis was also $\log_n$ transformed.

Analysis of differences between day and night feeding was done using a simple split plot ANOVA. The density effect was applied to the ponds and the day/night factor

was applied within a pond. Hence, between-pond effects and within-pond effects were split.

The question of whether total length or standard length is the better estimator of weight was answered using REML analysis models, fitted with error variances and associated degrees of freedom, which were then tested for differences using Bartlett's test for homogeneity. The error variance was then compared, as the same weight data was used for each length data set.

To determine the effect of food type (pellet vs natural food) on growth, a regression of weight on percentage of pellets in the gut was done.
The experiment was approved by the Far North Queensland Animal Ethics Committee, approval number FNQ- 01-01.

5.2.7 Experiment 5.3. Effect of broadcast feeding and probiotics on growth of golden perch in nursery.

Twelve thousand fingerling golden perch (SL 32·86 ± 4·29 mm, Wt 0·43 ± 0·196 g, n=50) were delivered by air transport from a commercial hatchery. On arrival fingerlings were treated for ectoparasites with 10‰ NaCl and 70 mg/l formalin for one hour. They were held in 2000 L tanks in a subdued light environment (average light intensity between 08:00 and 18:00 was 1·856 lux, see Chapter 3, Section 3.2.3 for explanation of light readings), fed frozen zooplankton blocks during an acclimation period of ten days, then weaned on to a formulated crumble diet (salmon starter 1 mm crumble, Pivot Co, Rosny Park, Tasmania) over a period of ten days. Five days after weaning was completed, fish were counted, then held for five days before introduction into cages in randomly allocated ponds. The fish were transported to the ponds in 10 g/L salt water and 0.02 mL/L Aqui-S, and acclimated by gradual mixing of pond and tank water for about 15 minutes before release into cages. Fish introduced into cages averaged 0.92 g weight, and each pond was stocked with 1,265 fish.

After three days in cages, when fish were avidly feeding on crumble food, they were released into the pond. Fifty fish were sampled every four weeks, commencing nine days after release into the pond. A random sample of twenty fish was collected every two weeks for gut analysis. The experiment ran for 81 days after release of fish. Six ponds were point fed at the end where the fish were released, and three ponds were fed at each end, with food distributed as widely as possible. Fish were fed three times daily (08:00, 12:00, 16:00), reducing to twice after 9 weeks. The 12:00 feed was stopped after nine weeks due to lack of food consumption at that time.

All data was analysed using the statistical package Genstat 6.1. To determine whether there was a significant change in growth over the three month period a split plot ANOVA was done where the whole plot stratum was the ponds and the split plot stratum the months. The sampling stratum was averaged over and the experimental units analysed directly (no transformations). A general ANOVA was done to compare average weight of broadcast vs point fed fish at the end of the trial.

To test for differences in size frequency distribution Kolmogarov-Smirnov 2 sample test was performed. Testing for differences in proportion of fish eating pellet in broadcast vs point fed treatments was done by χ^2 test on the frequencies of fish eating more than 25% pellet and those less. Assessment of difference in size of fish eating pellets or insects was plotted out and compared-fish eating pellet compared to fish eating natural foods.

Three of the six point fed ponds were treated with probiotics. Point feeding was chosen as the 'control' feeding treatment as it was used in the first trial. The probiotic product used was a commercially available microbial product (*Bacillus* spp.) specifically targeted to Penaeid prawn farmers (Platypus, International Health products, Blacktown, NSW) but promoted by the agents as being very versatile and useful in freshwater. It is a program of weekly treatments over a four-week cycle to introduce large volumes of beneficial bacteria into the pond water. They were administered according to the manufacturer's instructions (a shuttle program of four different bacterial preparations, administered weekly in succession). Growth of fish in treated and untreated ponds was compared. Statistical analyses were the same as those

detailed above for growth. Plots of pH indicated that variation was too great to permit meaningful statistical analysis.
The experiment was approved by the Far North Queensland Animal Ethics Committee, approval number FNQ- 08-01.

5.3 Results

5.3.1 Experiment 5.1. First Pond Nursery and Growout Trial

5.3.1.1 Growth

Growth was initially slow, but increased rapidly after about 120 days (Figure 5·1). This increase was in winter when temperatures were comparatively low (Figure 5·2). Average weight and length of the fish at day 221 (the last measuring period before the death of fish in one high density treatment -see survival) is presented in Table 5·2. Size frequency distributions after the last measure and weigh event are plotted in Figure 5·3. Final weight of fish in the remaining high density treatment was 120.68 ± 11.18 g at day 256, and the low density at day 256, 152.55 ± 14.85 g and at day 283 the final low density pond was harvested with an average weight of 183.19 ± 16.71g. These weights compared to the weights of the last complete measuring event indicate rapid growth in the last month before completion of the trials. Relative proportions of large fish increased dramatically with 35 days more of grow out (Figure 5·4).

ANOVA of weight at day 221 indicated a slight difference between treatments ($P<0.045$), and LSD indicated that the difference was one low density treatment which had significantly higher growth than all other treatments. ANOVA of condition indicated highly significant differences ($P<0.001$) but the LSD value is 0.00993. The difference again is between the highest performing low density pond (B3) with all other treatments being lower (Table 5·2).

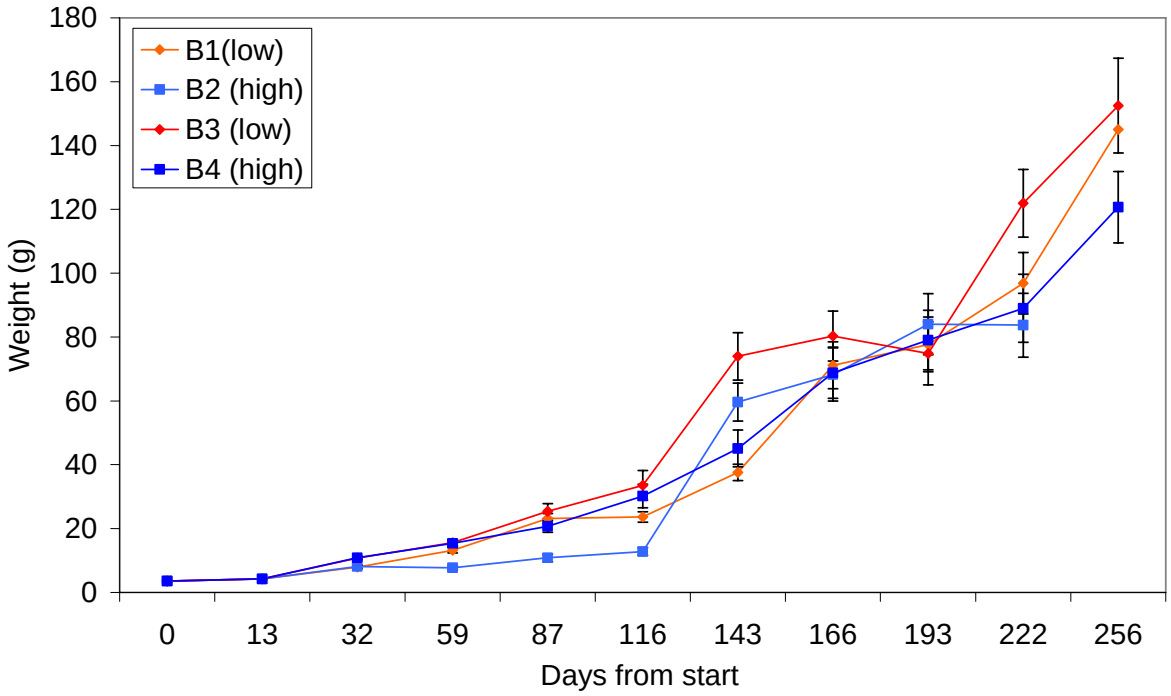

Figure 5·1. Mean (± SE) growth of golden perch in ponds at two densities, low density (105,000 fish/ha) or high density (31,250 fish/ha).

Table 5·2. Food conversion ratio, specific growth rate (as % increase per day), survival, mean (±SE) final weight and weight gain up to day 221 for golden perch at two densities in ponds.

	B1 (low)	B2 (high)	B3 (low)	B4 (high)	LSD from ANOVA
FCR	3.49	2.68	2.86	2.69	
SGR	1.495	1.429	1.599	1.457	
Survival (%)	99	98.86	101.8	94.85	
Weight at day 221 (g)	96.86 ± 9.62	83.75 ± 10.01	121.92 ± 10.61	89.02 ± 10.65	28.53
Weight gain	93.3	80.19	118.36	85.46	
Condition	0.2605 [bc]	0.2669 [ab]	0.2739 [a]	0.2535 [bc]	0.00993
% over 200g at harvest	11.87		18.66	26.01	

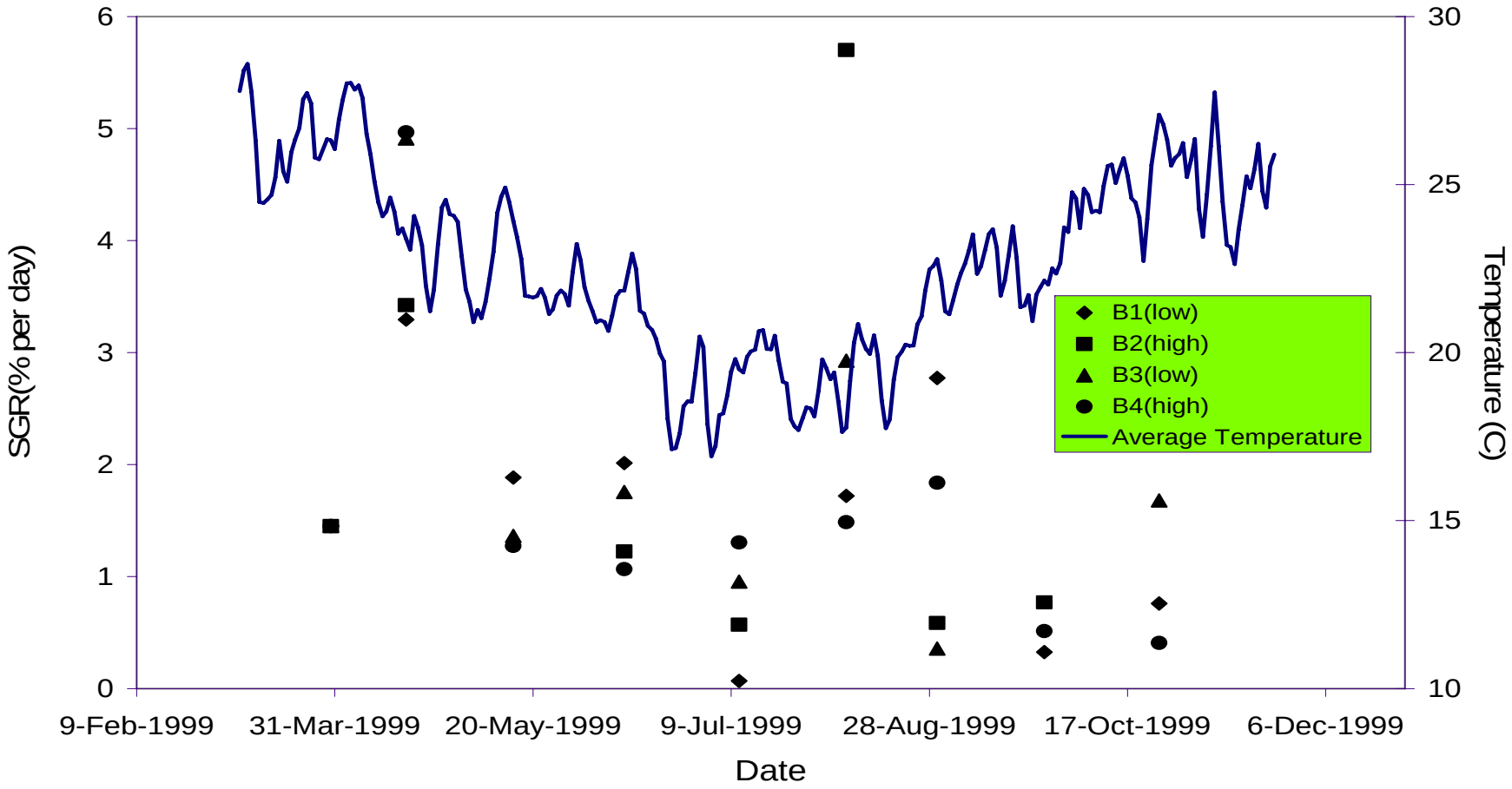

Figure 5·2 Specific growth rate and mean daily temperatures over the trial.

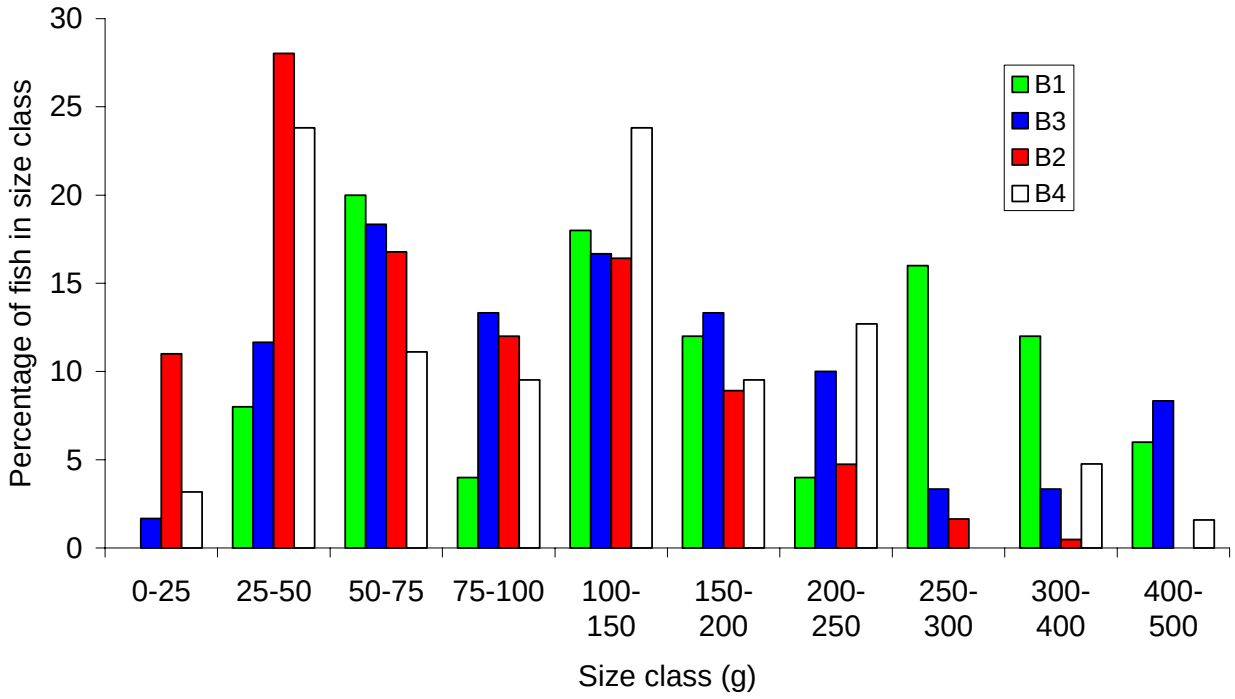

Figure 5·3. Size frequency distributions of golden perch at two different densities (B1 and B3 low density, B2 and B4 high density).

Size frequency distributions at day 221 demonstrated that the low density treatments had a higher proportion of fish in the larger size classes than the high density treatments (Figure 5·3) where it is apparent. The high density distribution is bimodal, with peaks in the 25-30 g and 100-150 g size classes, whereas low density treatments were closer to a normal distribution. Size frequency distributions appeared to be significantly different when two sample Kolmogarov-Smirnov tests were applied to compare pooled densities, including the measurement of all the fish that died in pond B2 as a sample. The pooled samples were significantly different at $P<0.001$ ($\chi^2=44.79$). The replicates for each density treatment were also compared to indicate likely differences in size frequency distributions. Both low density distributions tested as not significantly different ($\chi^2 = 4.08$, $p=0.130$), but the results of the comparison of the two high density distributions were highly significant ($\chi^2 = 11.40$, $p=0.003$).

Size frequency distributions at harvest found a very sharp increase in number of fish over 90 g weight after the 221 day period when lack of replicates precluded

meaningful statistical analysis. The decline in proportion of small fish in the final measuring periods is demonstrated in Figure 5·4.

The actual number of fish for the three size classes for grading at harvest were all within 10 fish of the prediction, for each size class, indicating that sampling was representative. Results of the grading are presented in Table 5·3.

Table 5·3 Size frequencies of golden perch from ponds at harvest

Pond	n harvested	% survival	% small (<90 g)	% medium (90-200 g)	% large (>200 g)	Days of grow out
B1	986	99	49.08	39.05	11.87	283
B2	3320	98.96	73.74	20.99	5.27	221
B3	1018	101.8	38.81	42.53	18.66	256
B4	3202	94.85	28.61	45.38	26.01	256

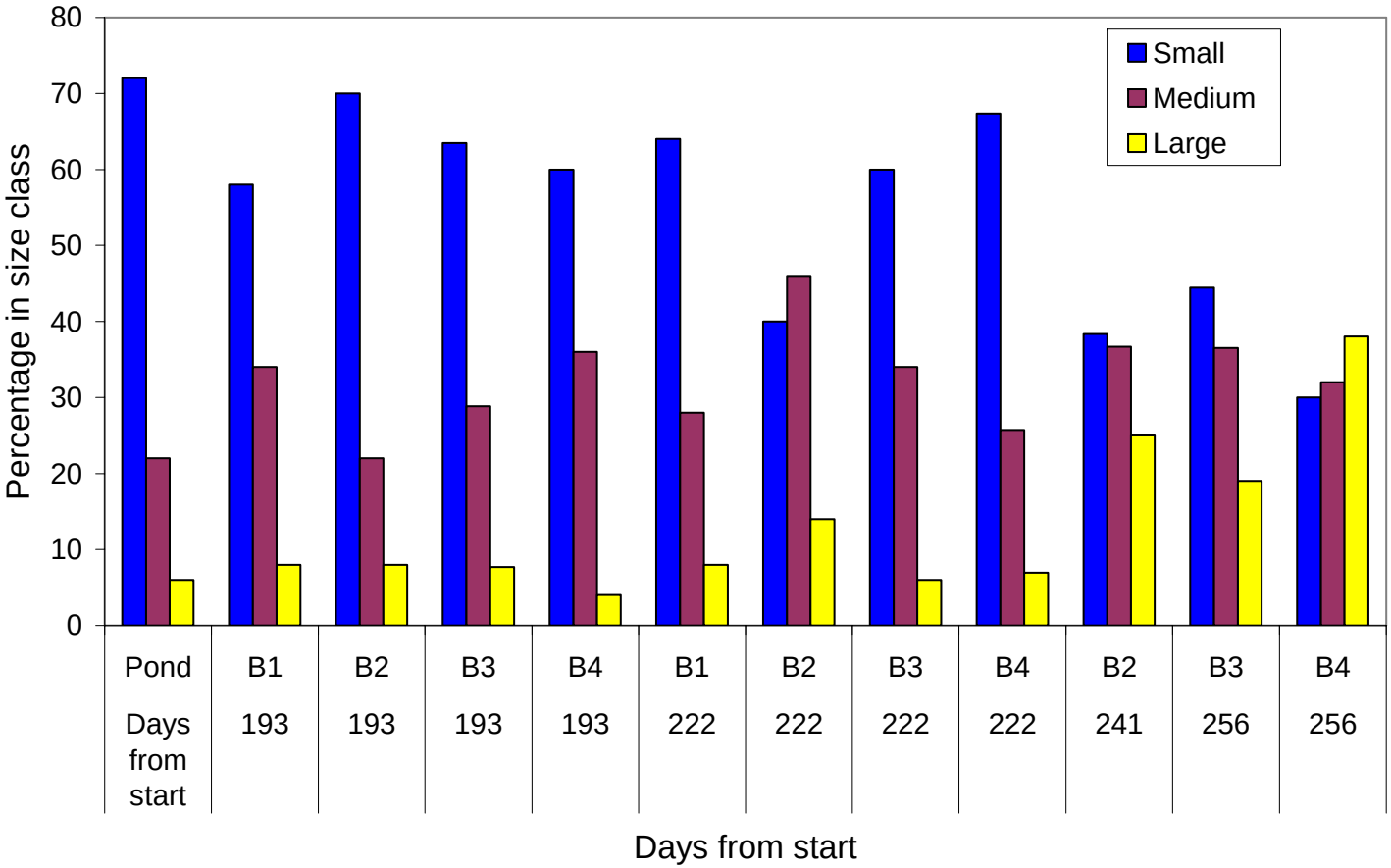

Figure 5·4 Size frequency distributions during the last three months of the initial growout trial. Proportions of larger fish increased immediately prior to harvest.

5.3.1.2 Survival

Survival was uniformly high over all treatments, varying from 94.8% survival in pond B4 to 101.8% in B3. Although fish were hand counted this high survival is due to counting error. The lower survival in pond B4 is due to a *Trichodina* outbreak, which killed about 20 fish. Fish in pond B2 died due to oxygen depletion (to 0.3 mg/L) after lightning strikes disabled power and backup systems during hot weather.

5.3.1.3 FCR.

FCR is high as all ponds were always fed to excess. It was found that water changes to reduce pH had a negative effect on fish feeding activity, reducing it until two or three days after the water change, which also contributed to the high FCR values. FCR is presented in Table 5·2.

5.3.1.4 Behaviour.

Fingerlings recommenced active feeding two days after introduction to cages in ponds. After one week free ranging in ponds the low density fish were observed feeding in a very tight school at the feeding tray, but only about 20% of the high density fish appeared to come in to feed trays while they were being observed. After about 30 days the water in all ponds was too green to see fish feeding.

5.3.1.5 Water Quality

Oxygen levels in the morning always exceeded 5mg/L. Temperatures are presented in Figure 5·2. The afternoon pH varied from 7.05 to 9.2, and water changes were done in ponds where pH exceeded 9.0 due to concerns about potential ammonia toxicity and effect of pH burning gills of young fish. Diurnal pH swings were less than 0.5 pH units.

5.3.2 Experiment 5.2. Nursery production of golden perch at two densities in ponds.

5.3.2.1 Growth at different densities

REML analysis indicated that the two densities had similar growth patterns over time (Figure 5·4), but there was a significant density effect ($P<0.001$). However, at the end of the trial the size frequency distributions were significantly different ($P<0.001$) between treatments. A larger proportion of fish in the low density treatment were

heavier than in the high density treatment (Figure 5·5). The smallest class (5-10 g) averaged 8% of the low density treatments, whereas in the high density treatments it was 46.67%. The next size class, 10-20 g, comprised 44% of the fish in low density, and in the high density 15.34%. Fish in the 20-30 g size class were more abundant in the low density treatment (Figure 5·6). At the other end of the scale, the largest size classes (>70 g) averaged 165.17 ± 10.69 g (14.11% of fish) in the low density treatment and 169.0 ± 13.04 g (17.33 %) in the high density treatment. The estimated transformed error variance of weights for high density was 1.095 and for low density it was 0.756 (147 df). Bartlett's test indicated that these variances were significantly different (P=0.025), the high density treatment being more variable than the low density.

Food conversion ratios (FCR), specific growth rate (SGR) and survival between ponds were similar (Table 5·4). Feeding was continually monitored using feeding trays, and adjusted regularly. After increases in feeding rate, there was usually a lag period of several days when there was more uneaten food than usual. Survival rate was high (>95%) in all treatments.

Table 5·4. Differences of size classes between high and low density treatments. Values are percentages, and are the mean of three replicate ponds. LSDs are derived from the raw data. Also presented are mean FCR, SGR and percentage survival of golden perch in nursery phase.

Size class	High Density	Low Density	LSD (5%)
<10 g (%)	46	8	16.35
10-20 g (%)	16	44	22.44
20-30 g (%)	5.3	15.3	8.28
FCR	3.3	3.8	
SGR	1.8	1.8	
Survival (%)	95.5	95.4	

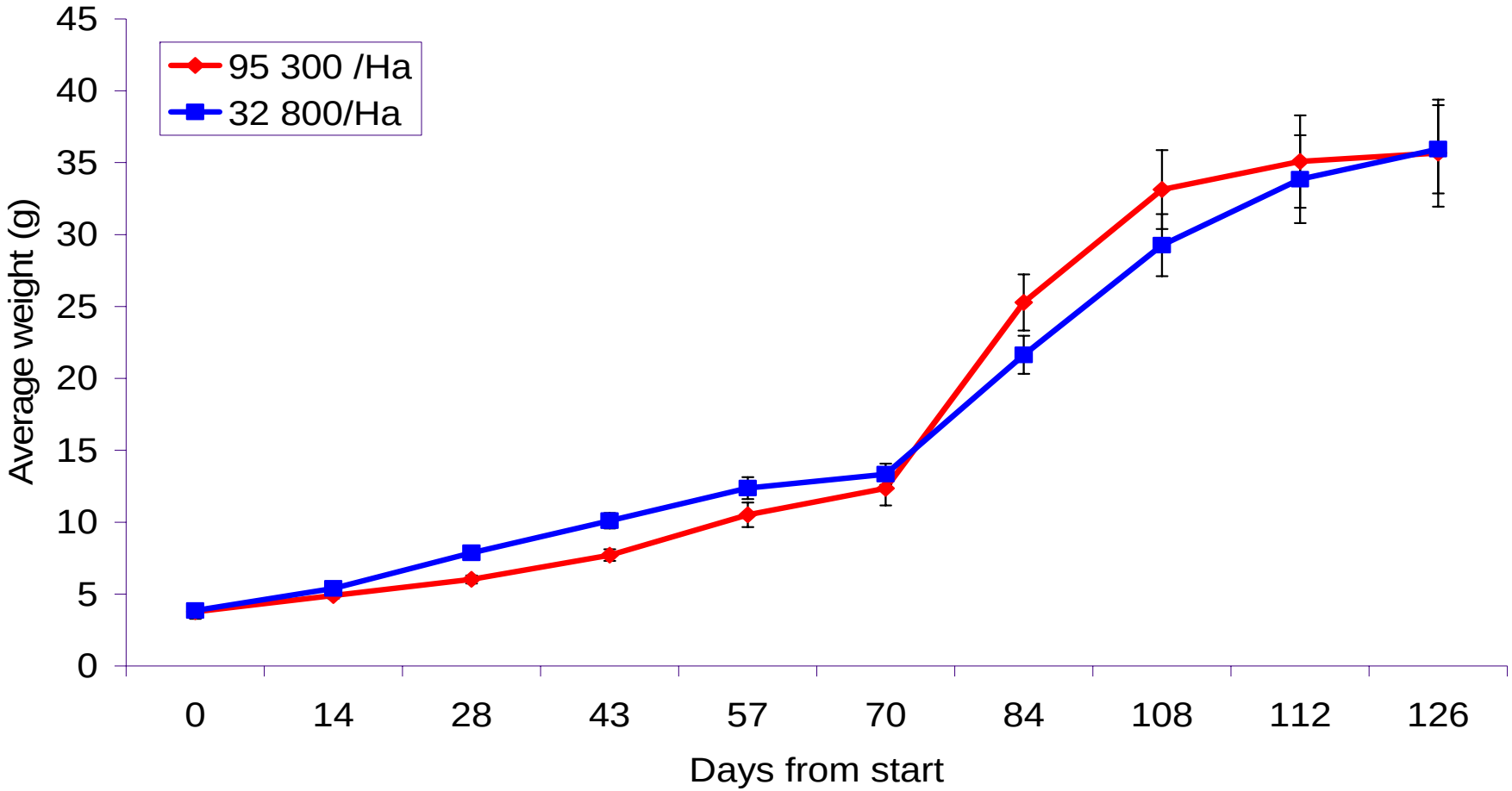

Figure 5·5. Growth of golden perch at two densities. Mean (± SE) weight (g).

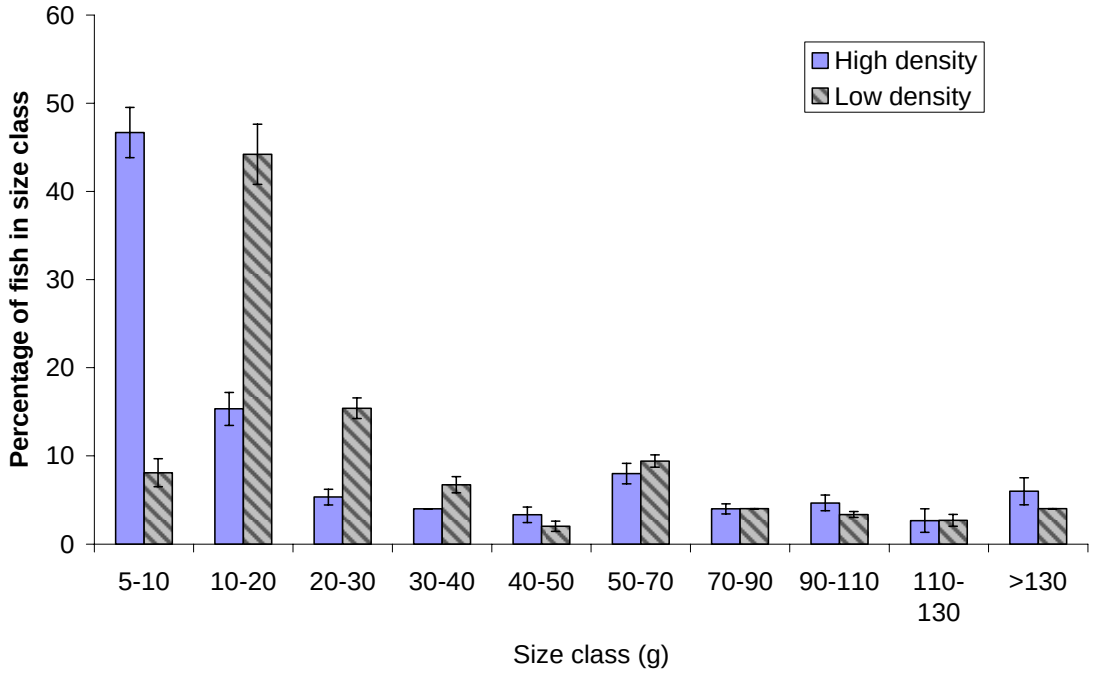

Figure 5·6 Mean (± SE) size frequency of golden perch, stocked at two different levels (High = 95,300 fish/ha; Low = 32,800 fish/ha) at the end of a 126 day pond-based nursery trial.

5.3.2.2 Reversion to natural production

During examination of stomach and intestine contents, no fish remains were found at all. There were small numbers of guppies (*Poecilia reticulata*), gambusia (*Gambusia holbrooki*) or rainbows (*Melanotaenia splendida*) in all ponds.

The proportion of fish initially eating pellets was high on average, but decreased over time (Table 5·5). It reduced immediately on introduction into ponds in the low density treatment, but declined more gradually in the high density treatment. When fish were eating pellets, the stomach was usually grossly distended and contained only pellets. The intestine contents were rarely 100% pellet, usually having indigestible parts of insects.

Table 5·5 Percentage of golden perch eating pellets (pellets comprised >25% of stomach contents) on each sampling occasion.

Date	HD Mean	LD Mean
08-Jan-01	76.6	36.6
22-Jan-01	53.3	38.3
08-Feb-01	47.47	35
20-Feb-01	30	45.96
06-Mar-01	28.3	31.6
19-Mar-01	38.3	38.3
02-Apr-01	65	53.9
17-Apr-01	41.6	36.6
01-May-01	33.3	29.6

The smallest size class of golden perch ate primarily chironomids or the freshwater cladoceran *Moina*, pellets being the third most eaten item. The second size class ate mostly Trichoptera. The remaining size classes ate mostly pellets. A significantly higher proportion of fish remained on pellets in the high density ponds (Table5·6).

Table 5·6. Mean percentage of fish sampled with over 25% of gut contents being pellets (P), Chironomids (C), Moina (M), Ostracods.(O), or Trichoptera (T). Mean is of all sampling events.

	Low Density					High Density				
Size class	**P**	**C**	**M**	**O**	**T**	**P**	**C**	**M**	**O**	**T**
1 to 5 g	27	40	40	2	2	53	65	38	5	15
5 to 10 g	13	27	3	2	30	27	13	12		15
10 to 15 g	23		2		5	25	2			
15 to 20 g	5					3				3
>20 g	8	2								

Initially, fish were observed to live under the shelter in a tight school. After about four weeks, most fish were observed feeding in a school, but with smaller fish at the end of the school. The fish at the head of the school fed in the mid-water column when the pellets were sinking. Active feeding was observed while the food was sinking, and

fish picked over the feed tray after all food had sunk. Feed tray monitoring indicated reduced feeding activity when pH was above 9.0. Additionally, feeding activity in the mid day feed diminished over time, and the evening feed was increased relative to other feeds to meet increased demand at that time. No significant difference was found in what fish had eaten at night compared to what they had eaten during the day. As the fish grew, size of natural food items increased. *Moina* (350-500 μm) was less common than chironomids and Trichoptera (3-5 mm) in the diet of larger fish. The Trichoptera were eaten in their cases, but the larvae were of similar size to chironomids. In the end of the period, corixid nymphs (2-3 mm) became a common food item, indicating that fish were large and agile enough to catch them.

Chironomid larvae, tubificid worms and glyptophysid snails were abundant on the feed trays. The most commonly encountered food items eaten by the fish were, in approximate order of incidence, chironomid larvae, chydorid cladocerans, trichopteran larvae (Leptoceridae), ostracods, copepods (calanoid and cyclopoid), corixid nymphs, gyrinid larvae, hydryphantid mites, snails (*Glyptophysa* (*Oppletora*)). A category called "others" covered baetid ephemeropteran nymphs (*Centroptilum* sp. and *Cloeon* sp.), terrestrial insects (one occasion) and unidentifiable material. Chironomids were the predominant natural food eaten by juvenile golden perch, followed by chydorid cladocerans. Stomach contents from the night samples contained primarily chironomid pupae, whereas morning samples only had larvae. All of the natural foods eaten, apart from chironomid pupae, cladocerans and copepods, are predominantly benthic. Odonata nymphs were abundant in the ponds but found in stomach contents on only two occasions.

5.3.2.3 Growth of fish on formulated or natural food.

Growth of golden perch eating pellets was significantly better than those feeding exclusively on natural production (Figures 5·7 and 5·8). There was no significant density-related difference in growth rate of fish that were eating the same foods. The more pellets fish ate, the faster they grew ($P>0.001$). The equation to the regression line is $Wt=13.89 + 0.6423*pellet\%$, i.e for every percentage point of pellet in the gut the weight was on average increased by 0.6423. As most golden perch eating pellets had gut contents > 85% pellet, on average pellet feeders grew 54% faster than fish eating only natural food.

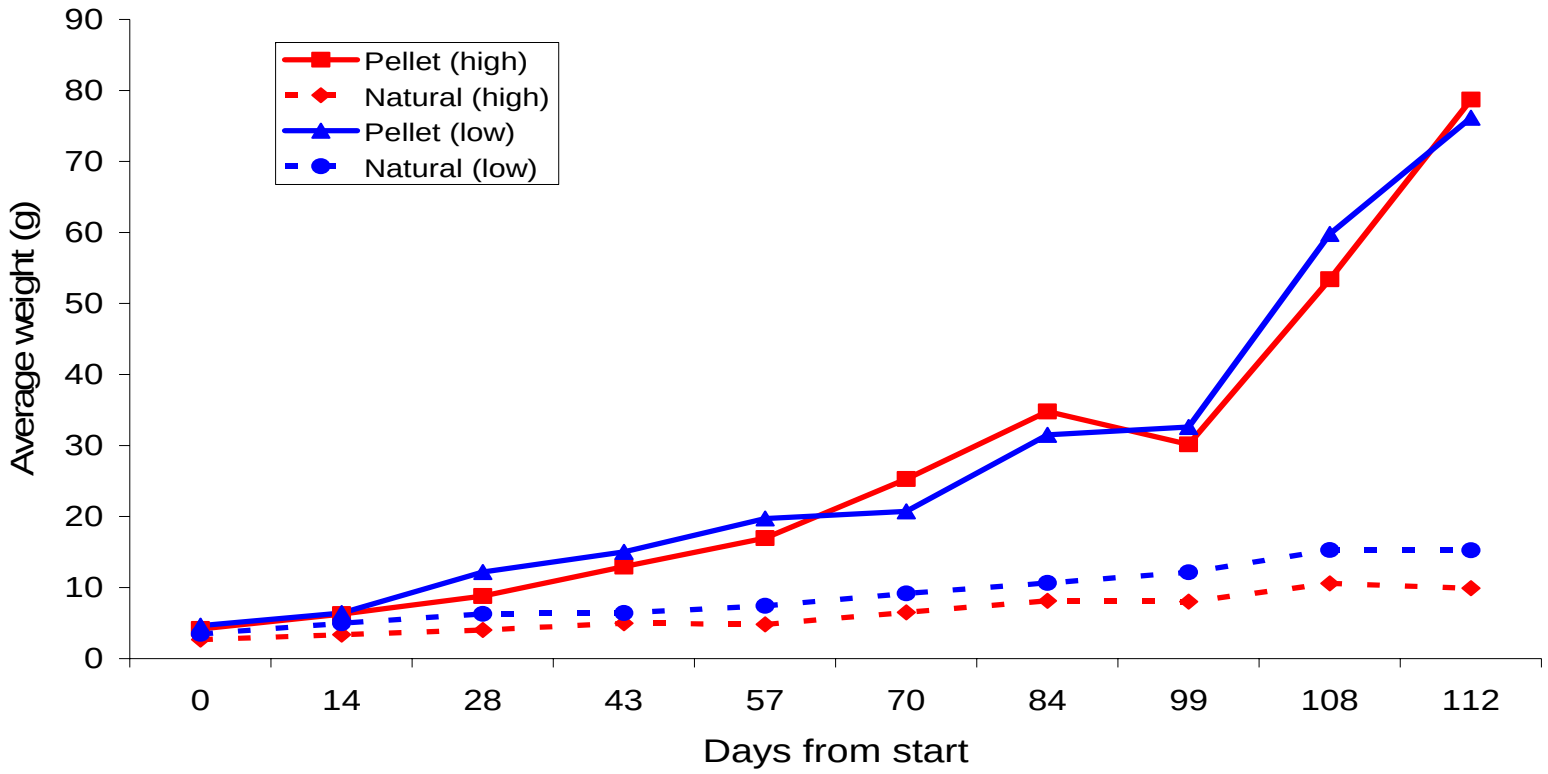

Figure 5·7. Change in mean (±S.E.) monthly weight of golden perch stocked at two densities (High = 95,300 fish/ha; Low 32,800 fish/ha) in pond growout. Solid lines represent growth of fish found with more than 25 % of gut contents as pellets, and the broken lines represent fish with less than 25% pellets (usually 100% natural food).

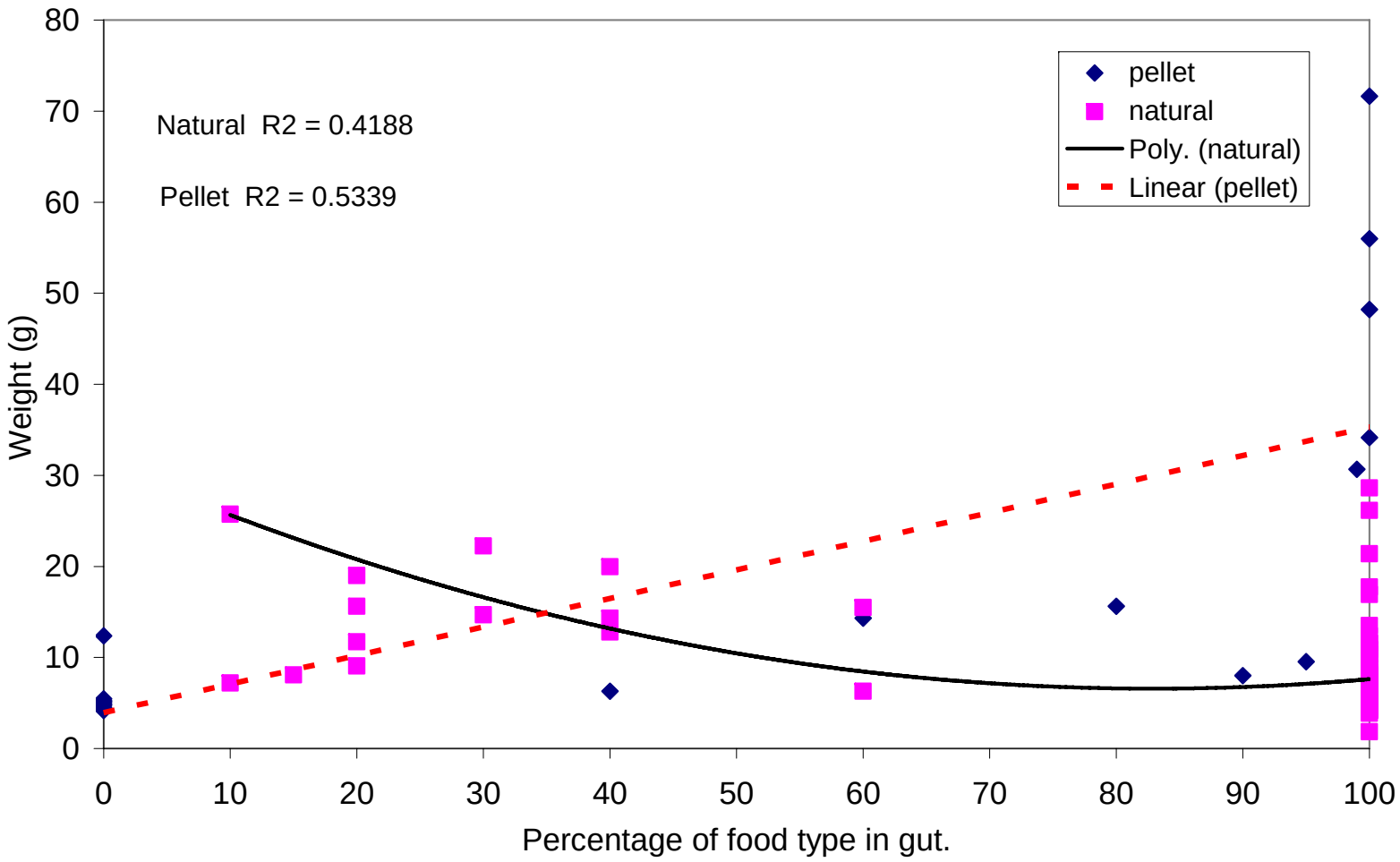

Figure 5·8. Scatter plots and trend lines of size of golden perch dependent on food type eaten (pellet or natural food).

5.3.2.4 Water quality

During the trial water quality was consistent across ponds. There was no significant difference ($P<0.05$) in oxygen, pH, temperature or conductivity between ponds. Oxygen varied between 6.98 to 16.7 mg/L (mean 10.8). pH varied from 7.49 to 10.01 (mean 8.93) at 14:00 to 15:00h. Diurnal variation of pH was never more than one pH unit; usually it was 0.5-0.7. Daytime temperatures varied from 24-32.6°C (mean 27.4°C) whereas temperatures logged hourly ranged from 17-32.6°C (mean 26.0°C) (Figure 5·9).

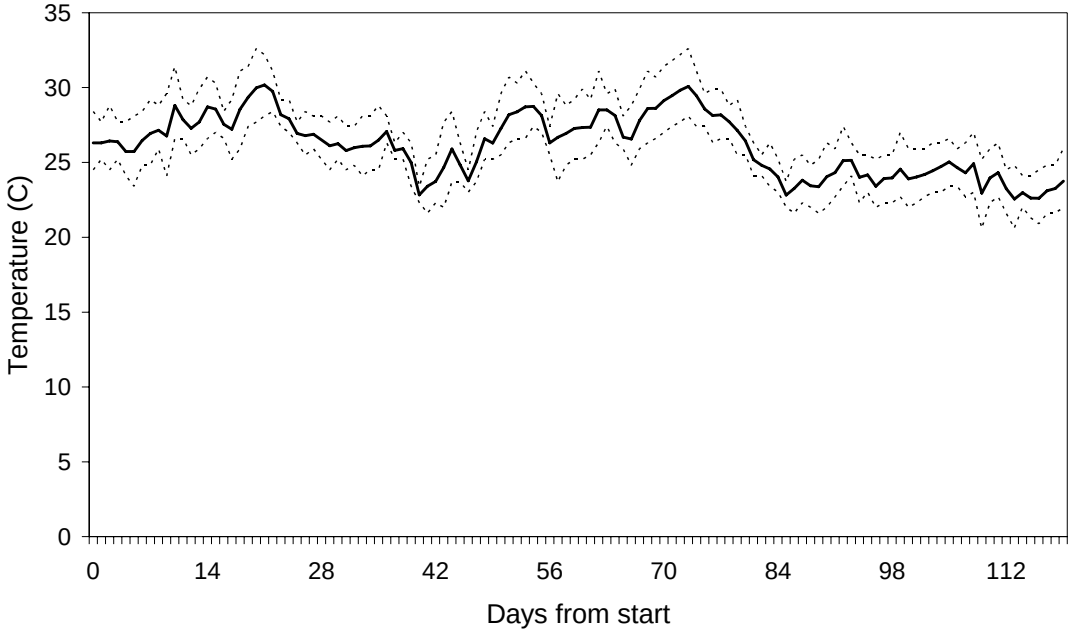

Figure 5·9. Mean, maximum and minimum temperatures from hourly logging data.

5.3.3 Experiment 5.3. Effect of broadcast feeding and probiotics on growth of golden perch in nursery.

5.3.3.1 Broadcast/point feeding

There were no significant differences in fish weight between comparable treatments until the final measurements were taken, at which time those fish that were broadcast fed had significantly higher weight than those that were point fed (Table 5·7). Those fish that were treated with probiotics did not differ in weight from those that were not subject to probiotic treatment and were point fed on any sampling occasion (Figure

5·10). The two point fed treatments (i.e. probiotic and non-probiotic) were subsequently combined for analytical comparison with the broadcast treatment.

Table 5·7. Mean (±SE) weights of fish in three treatments at each sampling period. Superscripts indicate significant difference at P< 0.05. LSD at 5% = 1.727 except when comparing within the same treatment, then LSD 5% = 1.456.

Treatment	Month 1	Month 2	Month 3	Month 4
Probiotics, Point fed	0.901± 0.02[a]	2.575 ± 0.31 [b]	6.718 ± 0.80 [c]	10.74 ± 0.52[e]
No probiotics, Point fed	0.899 ± 0.06 [a]	2.964 ±0.18[b]	7.046 ± 0.67[cd]	10.899± 1.14[e]
No probiotics, Broadcast fed.	0.975 ± 0.08 [a]	2.253 ± 0.16 [b]	8.607± 0.33[d]	15.639 ± 1.07[f]

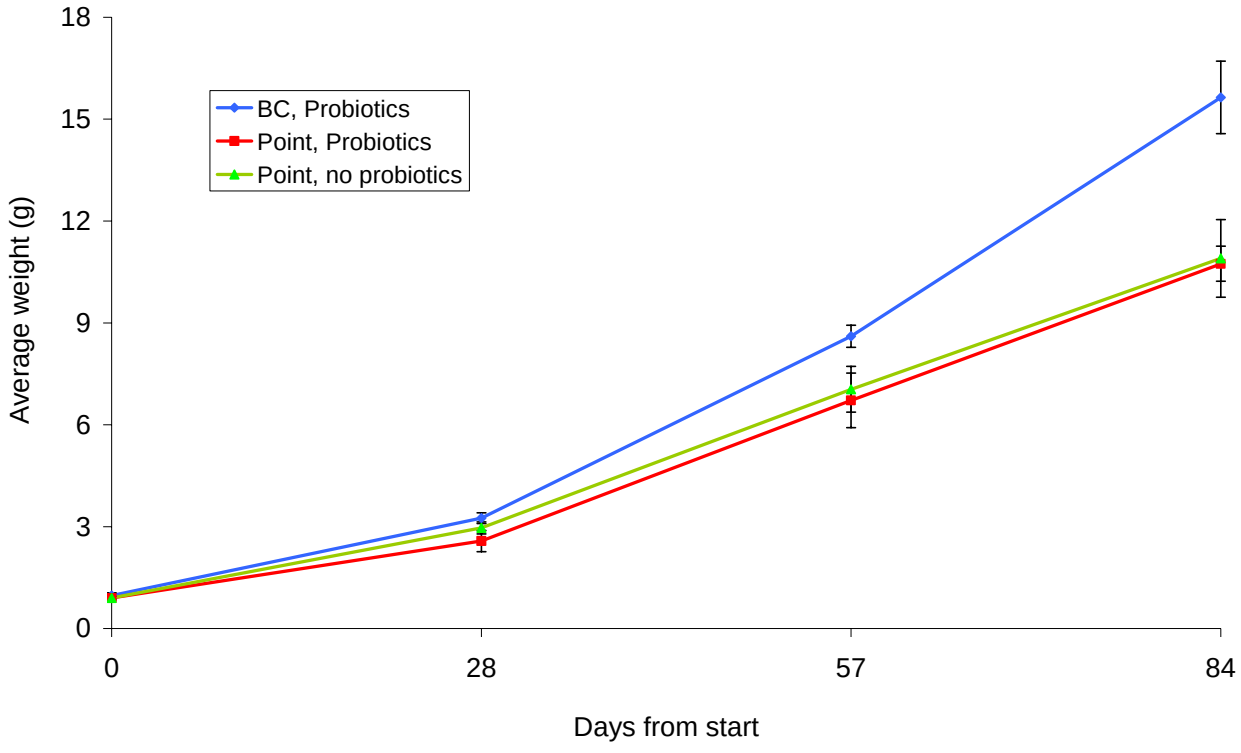

Figure 5·10. Mean weight (± SE) of golden perch in three treatments (broadcast fed and probiotics applied; point fed without probiotics, and point fed with probiotics applied) at each sampling period over three month nursery phase.

The test of difference of average weight at the end of the experiment using a general ANOVA and only comparing broadcast feeding to point feeding indicated a significant difference at F<0·05 (Table 5·7).

The Kolmogarov-Smirnov two sample test indicated a significant difference in size frequency distribution between broadcast and point fed ponds (at P<0·001) with a $\chi 2$ value of 43·32 on 2 df and sample size of 300. A significant difference in size frequency distribution was detected in all measuring periods.

The $\chi 2$ to test for differences in the proportion of fish eating pellets in broadcast or point fed treatments only found a significant difference in March. Those fish that contained < 25% pellets in their intestine had a size distribution skewed towards smaller size ranges, with a mode between 5 g and 10 g. Figure 5·11 indicates the shift in size classes, with broadcast treatment having more individuals in the larger size classes than the point fed. Those fish that were found to contain >25% of pellets in their intestine had a normal size distribution, with a mode between 15 g and 20 g (Figure 5·12).The retention rate on formulated food (>25% of gut contents pellets) at the end of the trial was 42.5% in the broadcast fed treatment, and 25% in the point fed treatment. χ^2 analysis of the numerical data gave a χ^2 of 5.43 (p=0.02) for retention on formulated feed in broadcast fed ponds in March, which is highly significant. However, in February no significant difference was detected (χ^2=3.68, p=0.055).

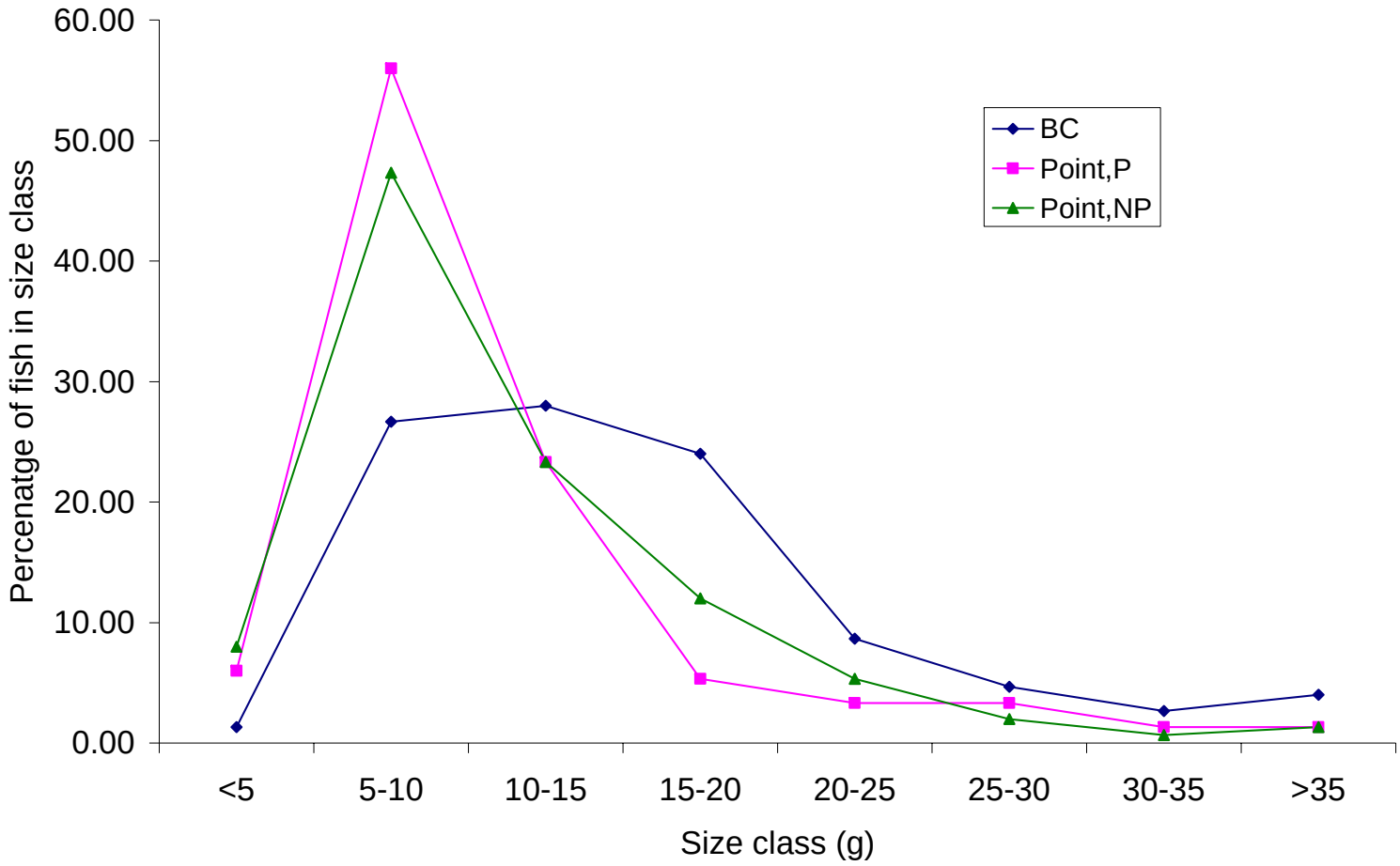

Figure 5·11 Size frequency distribution of fish feeding on pellets or natural foods at the end of the experiment.

5.3.3.2 Water Quality/Probiotics

There appeared to be no significant differences in afternoon pH (used as an indicator of algal activity) between ponds of any treatment. pH fluctuations were relatively consistent between treatments, and that no particular treatment displayed less variability than others (Table5·8). pH values were normally between 8-9. Secchi readings were not used as several algae (including *Microcystis*) at FFAC form flocs, which permit high secchi values but have a high impact on pH and other phytoplankton. Ammonia levels were measured in ponds with the highest pH once a week. However, the incidence of high pH and high ammonia levels was approximately equal in both probiotics and non-probiotic treated ponds. These parameters were too variable to permit any meaningful statistical comparison. Health of fish was not an issue over the sampling period. No mortalities from diseases were recorded.

Table 5·8. Mean (± SE)pH levels in probiotic and non probiotic ponds.

	Mean pH	Maximum pH	Minimum pH
Probiotics	8.66 ± 0.095	9.58 ± 0.095	7.31 ± 0.095
No Probiotics	8.62 ± 0.003	9.64 ± 0.003	7.40 ± 0.003

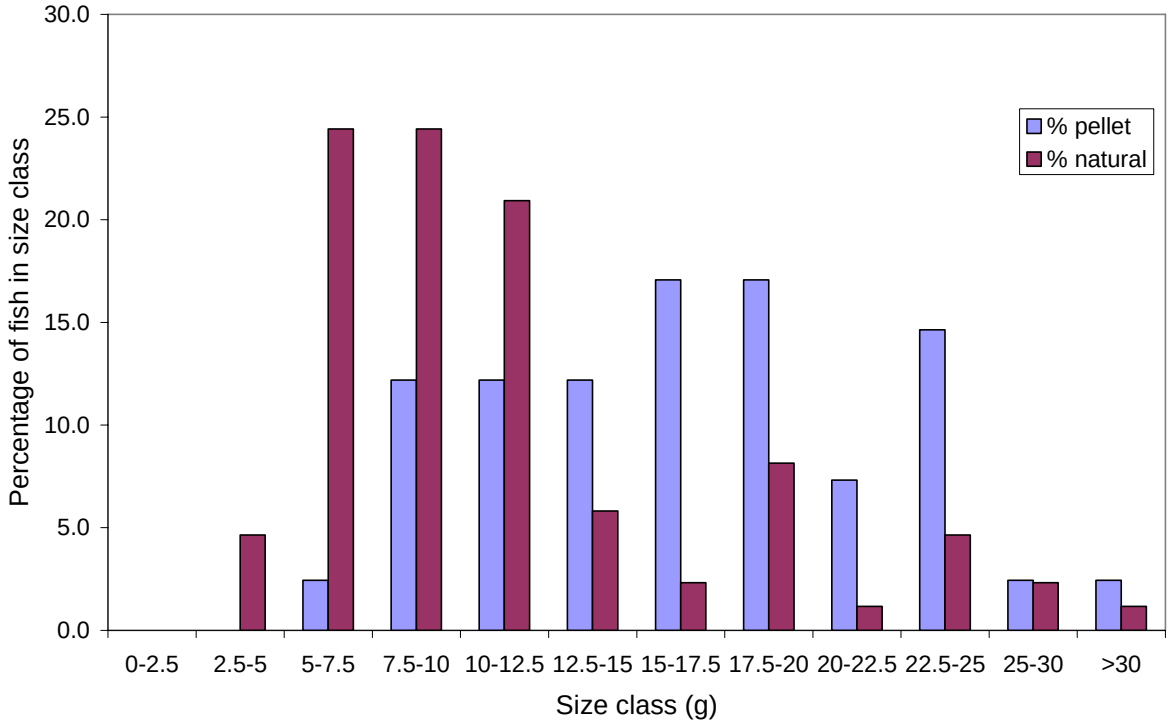

Figure 5·12. Size frequency distributions of fish at harvest after three months of nursery. All size classes are in grams

5.4 Discussion

5.4.1 Experiment 5.1. First Pond Nursery and Growout Trial

Growth of golden perch in this study is not comparable to the previous silver perch studies (Rowland, 1994; 1995b; Rowland *et al.*, 1995) due to the lower densities, different methods and start sizes used. However, Rowland's studies in ponds indicated that lower densities always produced higher growth rates, due to supplementation of formulated food by natural production in the ponds, although stomach contents were not examined to confirm this (Rowland pers. comm. 2002). The reason for the better growth rates in golden perch at low density need to be determined to ascertain whether it is behavioural, or due to food availability. Silver perch hierarchies are broken at high density (Harpaz *et al.*, 2001), and it is possible that the densities trialed here were not high enough to have this influence, as golden perch may develop feeding hierarchies (Stevenson and Grant, 1957).

Previous studies of golden perch in the wild have found growth over one year to vary from 160 mm (67-104 g) in rivers (Reynolds, 1976; Cadwallader and Backhouse, 1983) up to 227 mm(197-254 g) in an impoundment (Battaglene, 1991)(see Chapter One, Table 1·3). Weights are calculated from the correlations of Battaglene 1991. These calculated weights compare well with the weights obtained in this study for golden perch grown out over seven months (83-121 g). Golden perch growth under aquaculture conditions, using a diet formulated for another species, is comparable to growth of golden perch in natural conditions. Using a diet formulated specifically for golden perch could be expected to produce significant improvements in growth and FCR.

The extremely rapid rise in proportion of fish in medium and large size classes in the last month suggests that golden perch growth may be far better than indicated by average growth rates over seven months. As up to 26% of fish were of marketable size (> 400 g) after 10 months, which is comparable to other cultured species like barramundi, prospects for grow out of golden perch look promising. The reasons for this sudden surge in growth are unclear, but did continue after medium and large fish were restocked into ponds where they grew at over 100 g per month for another 6 months (Herbert, unpublished observations).

The SGR of juvenile (>3.5 g) golden perch over the experimental period was around 1.4 to 1.6%/day, which is comparable to that of other carnivorous fish such as Murray cod and barramundi of a similar or larger size (Abery *et al.*, 2002; Williams *et al.*, 2003). SGR is lower in larger fish (and higher in small fish) due to decreasing growth potential with increasing size (Jobling 1983), which explains the substantial reduction in SGR from the golden perch fry in the previous weaning experiments. The nutritional requirements of golden perch are currently not known, except that protein requirement is estimated at 40% (McFayden, 2001). SGR was lowest when temperatures were lowest but remained low towards the end of the trial when algal blooms required repeated flushing of ponds to keep them below pH of 9. This agrees with the findings of Stevenson and Grant (1957) that food consumption dropped below 24°C, and disturbance reduces feeding (and by extension growth).

Although significant differences in size frequency distributions for both densities were detected, they were due to one low density pond performing significantly better than the others. This indicates the need for more replication, to allow for the inherent variation in performance of individual ponds. Additional design improvement would be the use of more than two densities. However, due to reliance on air-shipped fingerlings, costs restrict the ability to overcome these limitations in design of pond based experiments on density effects.

The apparent bimodal distribution of the high density fish suggests a hierarchy based division in growth. The low density distributions appeared more normal but were still skewed towards smaller fish, although overall performance was significantly better than the high density fish. This pattern of higher proportion of larger fish in lower density also occurs in silver perch held at different densities (Rowland, 1994; Rowland *et al.*, 1994; Rowland, 1995b; Rowland *et al.*, 1995; McFayden, 2001). The lack of differences in average growth up until about 100 days after stocking suggests that high density nursery followed by lower density grow out, as done for silver perch, will be beneficial.

Survival in all treatments was high, despite the wide range in fish size (9 g to 473 g), indicating that golden perch are hardy and not cannibalistic. This is a significant

advantage over many other carnivorous species in which cannibalism is a serious problem (Parazo *et al.*, 1991; Baras and Jobling, 2002). Barramundi cultured in Northern Australia require frequent grading at least every week to reduce cannibalism losses up to about 100mm length (Tucker *et al.*, 2002), which imposes a significant handling cost. Predation by birds is a significant problem in previous experimental work on other native Australian fish (Rowland *et al.*, 2004). The very high survival rates demonstrate the efficacy of predator exclusion netting at FFAC. Recent developments in bird control indicate that exclusion may have to be the method of choice in future as disturbance or destruction of predatory animals is severely restricted by law, at least in Queensland.

FCR for golden perch varied from 2.7-3.5 and is an indication that feeding in this experimental situation was always to excess. It may also be an artefact of feeding a diet formulated for barramundi, a truly tropical, fast growing carnivorous fish. When fish stopped feeding (usually after water exchange or sampling) significant quantities of food were wasted. Golden perch are benthic feeders, so feeding rate could only be determined by using feed trays. As noted earlier, disturbance of fish reduces growth and food consumption considerably (Stevenson and Grant, 1957; Mélard *et al.*, 1996b; Ogle *et al.*, 2001). In aquaculture conditions management procedures (e.g. sampling to check size) would need to be low-disturbance methods, to minimise the impact on growth. Another factor in the high FCR is that the higher temperatures for more extended periods than occur in the natural habitat of golden perch may drive fish metabolism faster than might be normal in the cooler waters of southern Australia. Overfeeding due to selective feeding off feed trays was evidenced by presence of tubificid worms in the latter stages of the trial suggesting build-up of uneaten food on the pond bottom. The lack of significant differences between high and low densities treatments in FCR suggests that density effects at the levels trialed did not impact on FCR.

Stevenson and Grant (1957) commented on the high food intake of golden perch compared to other fish. Although their study is not directly comparable, the observation that golden perch could consume 6-7% of body weight or more per day (of fresh mollusc) over long periods of time indicates a high capacity for sustained feeding. Feeding ad libitum may not be the best way to feed golden perch as they have

long gut retention times if starved after feeding (96 hours)(Anderson and Braley, 1993) which could presumably lead to greater digestive efficiency. Some species of fish when fed below satiation rates grow equally compared to those fed to satiation (Lin and Yi, 2003). Research into timing and ratios of feeding could be beneficial in development of golden perch aquaculture, as in other species feeding régimes may be altered to reduce costs (Williams and Barlow, 1993).

Golden perch grow reasonably well, compared to growth rates in the wild, at low and high densities although growth was marginally lower at high density. The graph of growth suggests that growth rates start to differ at about 100 days (Figure 5·1), indicating a three or four month nursery phase at high density followed by a lower density grow out phase. The growth of golden perch in these trials are comparable to those of golden perch in the wild, suggesting that 12 months of pond culture will produce marketable (400-700 g) fish. In the current study 7-18% of fish were marketable after 7 months of grow out which suggests that there is high potential for genetic improvement of the species by selection of faster growing fish.

Further trials (Experiment 5.2) with more replication are necessary to determine whether growth is significantly better at low densities or whether the high growth rate in one of the ponds in this trial was abnormal.

5.4.2 Experiment 5.2. Nursery production of golden perch at two densities in ponds.

Nursery growth of golden perch at low density (32,800/Ha) and high density (95,000/Ha) was superficially similar. The larger fish ate predominantly pellets, and the smaller fish, insects and zooplankton. Nursery stage growth of silver perch was studied by Rowland (1994), at 25,000 and 80,000 per hectare. The coefficient of variation (standard deviation/mean) of silver perch (48·4 and 49·5) was lower than that of golden perch (79·2 and 72·3), indicating that the size variation of juvenile golden perch grown in this trial was more variable than exhibited in pond-reared silver perch. The uniform growth of silver perch is one of the characteristics making them desirable in aquaculture (Rowland and Barlow, 1991). Other species in

commercial culture are similarly variable, and some of this variability is related to parentage and larval history (Babiak *et al.*, 2004). This indicates promise for genetic improvement of aquaculture stock. The growth rates of silver perch and golden perch compare well, although with a less pronounced density effect in golden perch.

The high CVs for golden perch compared to silver perch indicate the grading of fish after a nursery phase is necessary. Grading after a four month period would split those fish which feed on pellets and those which don't, but the high CV of those fish that feed on pellets means that they would require further grading, into classes of rapid growers and slower growers at a later stage. Culture of golden perch in either cage or tank systems may be better than in ponds, at least in early stages, due to the problem of reversion to natural foods. Many other studies have found growth of fish to be better in cages (Rowland *et al.*, 2004), but this remains to be tested in golden perch. However, the benthic feeding habit of golden perch may not suit suspension in cages. Modular plastic raceways currently being used in southern Queensland for silver perch culture allow benthic feeding but restrict access to other benthic foods, so may be an answer to this problem.

High survival rates, the very wide disparity in sizes of fish, and the absence of fish in gut contents indicates that golden perch are not cannibalistic at this life stage. This allows benefits of reduced grading and potentially high stocking rates in an early nursery phase, a significant advantage over barramundi, a cannibalistic species requiring regular grading to reduce cannibalism (Parazo *et al.*, 1991). This offers significant advantages in high labour cost environments such as Australia, where grading can add considerable expense to fish farming operations.

The average growth rates of fish of all ponds rose rapidly in the final month, when water temperatures were falling. Water temperature may have influenced growth, as the normal range of golden perch is in higher latitudes, which have cooler water temperatures. Faster growth rates, linked to availability of food, have been observed when temperatures are falling in wild populations (Battaglene, 1991). Preliminary data also suggests that golden perch FCR is better in cooler (<25°C) temperatures than warm (>28°C) water (Herbert unpublished observations). Rowland *et al* 2004

found that the sympatric silver perch ceased growth when water temperatures exceeded 30°C.

Rowland (Rowland) believed that supplementation of pellets with natural food by silver perch reared in ponds contributed to the very low FCR. The size frequency distribution of golden perch at the two different densities in the present study indicated that the low density treatment had more fish in larger size classes than the high density. This may have been due to reduced competition for natural food resources in lower density ponds. It also emphasises the need for caution in interpreting mean values, as these indicated little difference between the treatments. The high FCRs in this study may be due to feeding a diet not formulated for the species in question, the overfeeding of the fish, and the higher temperatures than the fish is normally exposed to. Further investigations into the dietary and temperature requirements of golden perch are required to address this issue.

The schooling behaviour may permit development of feeding hierarchies, reducing exposure of some fish to pellets. Some smaller fish that were eating pellets may only have access to pellets that had been on the bottom for some time, thus reducing their comparative nutritional value due to leaching. The vast majority of fish that ate more than 25% natural food were in the smallest size class of 5-20 g. An apparent effect of more natural food availability in the low density ponds was the larger number of fish eating natural food in larger size classes. Another observation is that in low density ponds, larger size classes of fish on pellet food were relatively more abundant than in the high density ponds. This suggests that moderate supplementation of pellets with some natural food promotes faster growth as found with silver perch (Rowland *et al.*, 2004). As water quality in all ponds was not significantly different, food availability was probably the prime factor involved in the size frequency differences. This may have been related to foraging behaviour, which in at least some species is learnt (Kohda, 1994). Faster growing fish may have adapted feeding behaviour, which was advantageous in the pond situation.

In order to increase production of golden perch, improving the transition of fish into ponds to maximise retention on the pellet diet is therefore essential. The natural foods

that golden perch eat indicates a benthic foraging habit, which in turn indicates feeding sinking pellets to maximise contact with, and hence retention on pellets.

Fish eating natural foods do not incur a direct cost through use of pellets, but do incur costs through ammonia production and oxygen use. The percentage of fish eating pellets at the end of the nursery phase was about 30%. Improvement of the retention rate would improve growth rates and thus viability of golden perch farming. Broadcast feeding will increase exposure of benthic feeding fish to pellet food. Broadcast feeding was not tried previously because aggressive interactions were not observed in golden perch point-feeding in tanks. Point-feeding in ponds allows ease of observation and monitoring of food consumption. Broadcast feeding may also reduce hierarchies if they exist. The results of this trial suggest that broadcast feeding may assist in increasing consumption of pellets by golden perch.

The immediate reversion of the low density fish to natural production suggests either a difference in foraging behaviour developed earlier, or that development of hierarchies caused an immediate reduction in access to the pellet food. As natural food was abundant in all ponds availability of food *per se* was not a factor affecting higher early retention rates in the high density ponds, the schooling behaviour observed early in the trial may have been the reason for the higher retention rates in the high density treatment. The school of fish was far bigger and so moved slower, and the density of fish in the large school may have broken down hierarchies that could have formed in the lower density treatment. This would have restricted access of relatively more fish to the pellet food and thus they would have reverted back to feeding on natural production, either zooplankton or Chironomids.

Comparison of stomach contents and intestine contents demonstrated that pellet feeders supplemented their diet with benthic insects, particularly chironomids, overnight. Odonata nymphs were only found on two occasions, even though they were abundant in the ponds. This suggests more selective feeding in the aquaculture ponds than has been found in natural environments (Barlow *et al.*, 1987; Battaglene, 1991). Sampling of the natural food in the pond was not attempted, as macroinvertebrates are notoriously patchy in their distribution (Stewart and Loar,

1993), and repeated sampling may have disturbed fish. Regular disturbance has a negative effect on fish growth (Mélard *et al.*, 1996b; Ogle *et al.*, 2001).

As the fish grew, size of natural food items increased. *Moina* (350-500 μm) was less common than chironomid larvae and Trichoptera (3-5 mm) in the diet of larger fish, probably due to increase in gape and ability to ingest larger prey. By the end of the trial, corixid nymphs became a common food item, indicating that fish were large and agile enough to catch them. The smallest fish ate *Moina* over the whole nursery period, suggesting that zooplankton is not the optimum long term diet. It suggests that golden perch growth may have been restricted by factors other than diet, as mass chironomid and ephemeropteran emergences were observed periodically throughout the experimental period.

The consumption of chironomids and trichopterans indicate a benthic feeding habit. Management of plankton and detritus to minimise natural production, as well as modifying pellet feeding strategies (diet formulation, feeding frequency, attractants, timing etc) will be essential to maintain pellet feeding behaviour in pond reared golden perch. Minimising production of zooplankton may be possible by control of algae, but control of detritivorous Chironomids may be far more difficult. Introducing fish immediately after filling may lessen the availability of chironomids in the short term, although adults appear to be attracted to oviposit in newly filled ponds and the progeny pupate after about 3-4 weeks. Management strategies to reduce natural production, at least in the nursery phase, should ensure better retention and consequently better growth rates. Culture structures crowd fish together and minimises access to natural foods. Preliminary results on golden perch in raceways produced much faster growth rates than these trials (McVeigh, personal communication, 2002), and may be one way of maintaining pellet feeding behaviour.

Pellets appear to be digested very quickly. Observations of intestinal contents after morning feeds found pellets in the distal portion of the intestine and natural food in the anterior part, suggesting about a 12 hour residence time for food in fish which are regularly fed. Anderson and Braley (1993) found a residence time of 96 hours in golden perch which were starved after the test meal. Thus, it appears that regularity of

feeding influences gut residence time, and could potentially be manipulated to increase digestive efficiency.

There was no apparent difference in the types of food eaten by golden perch at different densities. Chironomids and Trichoptera were the favoured food in all ponds. In ponds where more than half of the zooplankton was copepods, *Moina* was the predominant zooplankton eaten by the fish, indicating selective feeding behaviour. The results of this work suggest that an initial nursery phase at high density will increase retention of golden perch on pellet food. A three to four month, high density nursery phase is indicated, to increase the proportion of fish retained on pellet food. At the end of this period, grading of fish into those that perform under aquaculture conditions and those that do not will select for faster growing, pellet feeding fish. This will have substantial impacts on future grow out.

5.4.3 Experiment 5.3. Effect of broadcast feeding and probiotics on growth of golden perch in nursery.

5.4.3.1 Broadcast/Point Feeding.

Broadcast feeding had a positive effect on growth, size frequency distribution and retention on pellet food at the end of the experiment period. Broadcast feeding in the nursery phase resulted in a significantly greater final weight than point feeding. There was a marginal difference (although not significant at F=0·05) in the third month. This suggests that growth differences are not established until fish reach an average weight of 6-8 g. This may be the point at which schooling behaviour influences food availability. Observation of schooling behaviour in point fed ponds indicated that larger individuals arrived at the feeding tray, fed, and then moved on. Smaller fish at the end of the elongated school therefore had little access to the formulated food, and were not able to feed on it. In broadcast fed ponds, the smaller fish were more able to access the broadly dispersed food while still in the school. Point feeding may only feed 50% or less of silver perch in ponds (Rowland and Walker, 1995).

Fish feeding on natural food did not grow as fast as fish eating formulated food, and more fish eating pellets were in the larger size classes. It is likely that social factors in

the nursery phase in aquaculture ponds affected access to formulated food and thus retention rates. Broadcast fed fish had a far higher rate of retention on formulated feed suggesting that there may be social interactions in the pond environment which influence retention on formulated food. Possibly the wider spatial availability of the food permits greater retention on formulated food. As found in the previous study, retention on pellet is important in growth of fish in aquaculture ponds.

The size frequency distribution of fish in broadcast fed treatment was more even than that in point fed. The broadcast fed treatment frequency distribution was significantly different from point fed, the distribution not as skewed to small fish. Broadcast fed ponds had lower numbers of fish in the smallest two size classes, and more in all of the larger size classes. This corresponds with the previous results where fish eating formulated foods were larger overall than those on natural food, and reflects the beneficial effect of broadcast feeding in higher retention rates on formulated food.

The results of this experiment indicate that in some aspects golden perch are similar to silver perch and that management of both species requires careful monitoring of food consumption (Rowland and Walker, 1995; Rowland *et al.*, 2001).

The results of this trial also indicate that there is still a significant 'tail' of fish that do not grow rapidly. Even though broadcast feeding increased the proportion of fish eating >25% pellet substantially, there were still more than 50% of fish in broadcast treatment reverting to natural food and hence not growing as rapidly. Food availability was not the issue in broadcast fed treatments. It may be that the size or age of transition into ponds after grading is a critical period. Certainly larger fish graded after the nursery phase and re-introduced into ponds resume eating pellet food. Possible reasons for reverting back to natural food even in broadcast fed treatments are that the current fish food is not as palatable as natural food, or that there are a proportion of fish that just do not grow due to genetic or other intrinsic factors. The observation that a small proportion of fish on natural food do grow to similar size as those on pellet suggests that the natural food itself is not inadequate, but the growth in the fish is restricted by other factors.

5.4.3.2 Probiotics

The results of this study concur with other studies on probiotics which were inconclusive regarding effect on water quality or fish health (Queiroz and Boyd, 1998; Gomez-Gil *et al.*, 2000; Shariff *et al.*, 2001). The intrinsic variability of the pond system limits the potential to quantitatively define whether probiotic products have an effect on water quality.

The application of probiotics did not significantly affect either pH or ammonia levels. Afternoon pH levels were too variable to permit statistical analysis. The probiotics were designed for disease (*Vibrio* sp. and virus) inhibition, and water quality improvement, in prawn aquaculture applications, although we were assured that they were also suitable for freshwater. However, the probiotic bacteria used are reported to work best at pH of 7-8, whereas the pH for most of this trial was usually 8.5-9.

5.5 Conclusion

The results of these nursery trials indicate that adopting specific management practices of golden perch fingerlings is a high priority, which may be the reason why previous attempts at growing them have met with little success. Golden perch appear to require different feed and pond management compared to silver perch, particularly regarding feeding, access to natural food and retention of fish on formulated food. Management of ponds to promote natural production is probably detrimental to retaining golden perch on formulated foods, which results in poor growth rates.

Growth of the fish in the first trial was far better than the other trials (Figure 5·13). The growth of fish in the poorest performing nursery trial may have been due to unidentified stressors which predisposed the fish to *Tetrahymena* infection. This is discussed further in Chapter 7.

The major difference in treatment of fingerlings between the trials was the food offered. The food offered in the first trial was a diet formulated for Murray Cod, an sympatric Percichthyid, for which nutritional requirements have been quantified (De Silva *et al.*, 2000). The food offered in subsequent trials was barramundi grower

pellet, formulated for a tropical, euryhaline carnivorous fish. The first batch of fish was grown out in larger ponds to market size after the initial trial, on the Murray cod grower pellets, and grew at rates of >100 g per month (from 100 g+ to 600 g - 1·4 kg in 4 months). This strongly suggests that the food used in subsequent trials was not the best food available. However, it was the only food produced at that time which reliably sunk. Floating foods were tried on several occasions and always floated into the pond shallows and were not eaten by the fish, even when feeding rings and other methods were employed.

When growth of golden perch is plotted taking into account the season of stocking it becomes apparent that growth tends to increase rapidly during the late winter and early spring (Figure 5·14). This suggests that the tropical temperatures experienced at FFAC may be too high for golden perch to achieve maximum growth efficiencies. Preliminary temperature trials in indoor tanks with fingerlings found that at 20°C fingerlings grew faster and ate only about 60-70% of the food eaten by those maintained at 30°C. The growth trials suggest strongly that cooler temperatures for a longer period, and a better diet, would improve growth of golden perch substantially. This has important implications for culture of golden perch in inland waters, which are often turbid and remain relatively cool throughout the warm summer months. Temperatures in ring tanks (large pumped water storages of flood harvested water) in southern inland Queensland rarely exceed 25°C, or decrease below 12°C (ring tanks represent a significant potential aquaculture resource for floating cages or raceways). It is probable that somewhere in these boundaries is the optimum temperature for growth of golden perch.

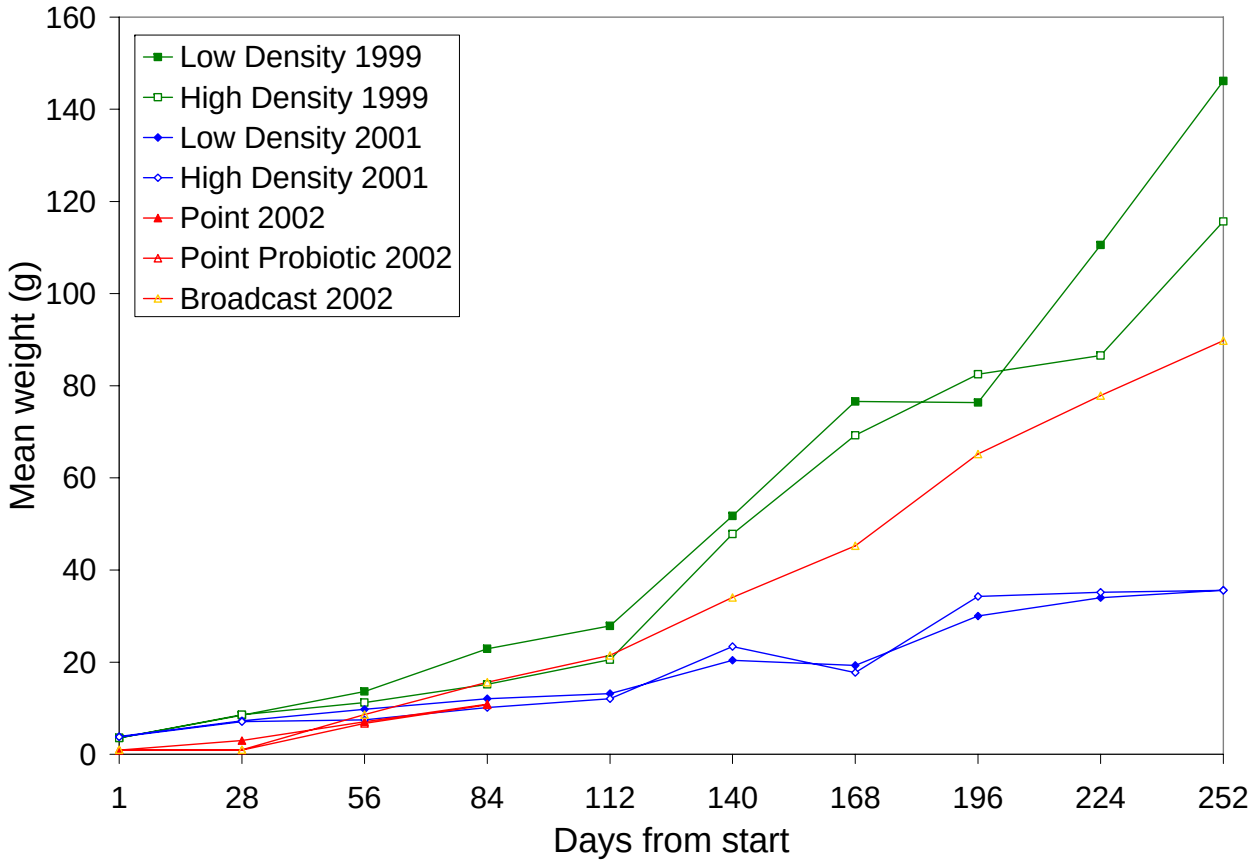

Figure 5·13. Growth of golden perch in different nursery trials. No account is made for differences in seasonal influences due to starting times. The 1999 trials were fed different diets to the others. Experiments in 1999 are experiment 5.1, 2001 is experiment 5.2 and 2002 is experiment 5.3.

Growth of juvenile golden perch in nursery trials in pond culture was not affected by density. This suggests that high density culture for golden perch could be viable, at least in the nursery phase, to reduce labour and other handling costs. Extremely high density may inhibit growth, as demonstrated in Chapter 4. These densities would be unattainable in the long term in pond culture due to water quality deterioration. The growth rates achieved suggest that a diet designed for golden perch, and maintenance in temperatures conducive to growth, could improve the growth rates and FCRs considerably. For further growout however, separation of those fish which have reverted to natural production and do not appear to grow will have benefits in terms of management. The lack of differences between growth, pellet retention and survival in two densities bodes well for intensive production of golden perch.

However, in many other species direct and indirect interactions can retard growth of some individuals in the population. To determine whether this is the case with golden perch, growing out the smallest fish separately should indicate if there are behavioural factors at play or whether restricted growth is due to another factor. It will also indicate the growth potential of smaller fish.

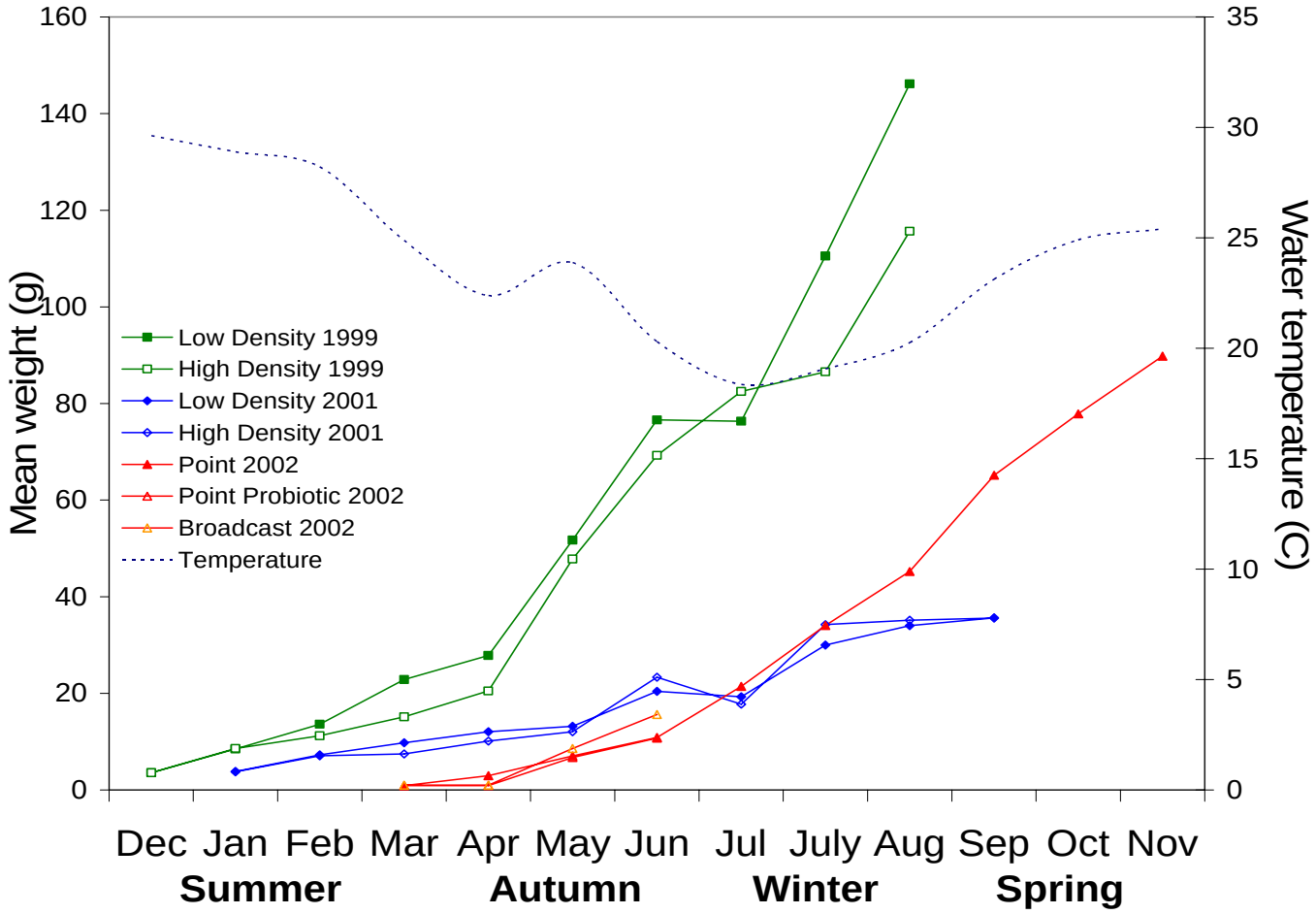

Figure 5·14. Growth of golden perch accounting for season of stocking. The 1999 trials were fed different diets to the others. Experiments in 1999 are experiment 5.1, 2001 is experiment 5.2 and 2002 is experiment 5.3.

Chapter 6
Growout of graded and ungraded golden perch, and sexual dimorphism, in pond culture.

6.1 Introduction

Size grading of fish in aquaculture is a commonly used management tool to assist in ease of handling, feeding and harvest. In many species of fish, grading has a significant effect on growth rates, due primarily to development of dominance hierarchies that influence access to food, food conversion efficiencies, or sex (and hence growth) of fish (Francis and Barlow, 1993; Goudie *et al.*, 1993; Harpaz *et al.*, 2001; Kestemont *et al.*, 2003; Saillant *et al.*, 2003). Conversely, there are numerous studies which indicate that grading has little or no effect on growth rates (Lee, 1988; Wallace and Kolbeinshavn, 1988; Baardvik and Jobling, 1990; Sunde *et al.*, 1998; Lambert and Dutil, 2001; Panagiotaki *et al.*, 2001; Martins *et al.*, 2006). Grading is of particular importance in species that are cannibalistic (Parazo *et al.*, 1991; Mélard *et al.*, 1996a; Qin and Fast, 1996; Baras and Jobling, 2002; Kestemont *et al.*, 2003). In species in which cannibalism is not important, grading may be important if densities are not sufficient to break down behavioural impediments to homogeneous growth (Harpaz *et al.*, 2001).

The large tail of slower growing golden perch (over 40% of fish stocked) is of concern to development of culture of this species. Although early weaning and grading of post-nursery phase juveniles is possible, it is desirable to determine if slower growing fish are in fact inhibited in growth by larger, dominant fish. If this is so, sequential grading through the grow out phase would be a viable technique of improving productivity and overall growth rates. Sequential grading is practised in channel catfish culture in the United States (Silverstein and Freeman, 2001). Previous studies of density and growth of golden perch suggested that a proportion of the reduced growth may be due to behavioural factors, although this was not clear. The

success of some fish apparently feeding exclusively on natural food (at least at the time of sampling) suggests that other factors may be at play.

Separating smaller fish from larger fish of the same population should provide an indication of whether dominant larger fish are inhibiting growth or whether genetic or other factors are more important (Wohlfarth and Moav, 1994; Endemann *et al.*, 1997; Martins *et al.*, 2006). Certainly genetic factors are of great importance, as illustrated in an exhaustive and complex study on European sea bass, which indicated that parentage had a major impact on growth rates, sex ratios and heterogeneity (Saillant *et al.*, 2003). Parentage is also important in determination of sex ratios and growth rates in channel catfish (Goudie *et al.*, 1993). Intrinsic differences in feeding behaviour of smaller and larger fish, independent of intraspecific interactions, may also affect growth rates in some species (Martins *et al.*, 2006).

Sexual dimorphism is a characteristic important in fish culture, as in many species one sex grows faster than the other. For example, European sea bass females grow larger than males (Carillo *et al.*, 1995; Saillant *et al.*, 2001) leading to much interest in development of monosex culture. Similarly, Eurasian perch females grow faster than males (Malison *et al.*, 1988; Juell and Lekang, 2001), as females eat more and have better FCR than males (Malison *et al.*, 1988). Golden perch are also sexually dimorphic, females being significantly heavier for given length than males (Battaglene, 1991). Length weight relationships for golden perch were:
For females: $W = 1.155*10^{-5} * L^{3.07}$
For males: $W = 2.496*10^{-4} * L^{2.55}$ (Battaglene, 1991, p 60).
Battaglene states that "females were heavier at any given weight than males…" (p60), but the equations as presented indicate that a 200mm female would weigh 134g and a male 184g. From the text and the graphs these equations are incorrectly attributed and for table 6.1 are reversed, i.e. the female equation as in the thesis used for males, and vice versa.
Growth curves (von Bertalanffy) for golden perch indicated faster initial growth (measured as length) in females, with males catching up at about 2 years age (Table 6·1). This has implications for aquaculture, as according to the length weight equations after one year females were about 180 g and males only about 90 g (Table 6·1). At the end of two years they would both average 700 g. It is important to

compare the growth rate of male and female golden perch in a culture situation, to ascertain if it matches that of the situation in a natural environment, and also to compare sex ratios. If there are significant differences in growth rates between the sexes in culture conditions, modification of sex ratios could be a management option to improve productivity of golden perch.

Table 6.1 Weights for golden perch for wild caught golden perch (Battaglene, 1991). Refer to text for the equations.

THIS TABLE HAS BEEN REMOVED DUE TO COPYRIGHT RESTRICTIONS

It is possible that apart from genetic factors, abiotic factors may be important in sex determination, and these are substantially different in a culture environment. Temperature and density are two factors important in sex differentiation of fish (Roncarati ***et*** al., 1997; Baroiller, 1999), meaning that timing of spawning or intensive holding of golden perch fingerlings in hatcheries may influence sex ratios. Parentage of fish is also important in determination of sex ratios (Goudie ***et*** al., 1993; Saillant ***et*** al., 2001). Saillant ***et*** *al* (2001) found that particular parentages produced differing sex ratios, which could have dramatic effects on sex ratios in a culture situation. Often golden perch used in culture come from limited parentages due to the large numbers of fingerlings produced from a single spawning (over 200,000).

Thus, it is important to gain an understanding of the effects of sexual dimorphism and sex ratios in the culture context, to determine whether manipulation of sex ratios may improve production characteristics.

6.2 Materials and Methods

Juvenile golden perch from nursery trials of point and broadcast feeding (Experiment 5.3) were used for this trial. In that experiment, six ponds of 1,265 fish were point fed

and three were broadcast fed. There were no significant differences between replicates of each treatment. After the 84 day nursery period, fish from three point fed ponds and three broadcast fed ponds were harvested, and graded with assistance of an adjustable bar grader into large and small halves for each pond. These fish were then restocked into separate ponds. The distribution and mean starting size of fish is presented in Table 6·2.

Table 6·2 Mean (±SE) weights (g) and history (feeding treatment in nursery), and number of replicate ponds of golden perch fingerlings in this trial.

	Point fed		Broadcast fed	
	Replicates at start	**Mean weight**	**Replicates at start**	**Mean weight**
Small	3	6.21 ± 0.42	3	10.02 ± 0.25
Large	3	15.68 ± 0.20	3	21.38 ± 1.69
Ungraded	3	10.9 ± 1.14		

In the week after restocking, mortalities due to a *Saprolegnia* sp. fungal infection in the caudal peduncle occurred. This was associated with the grading and resulted in significant mortalities, which resulted in uneven numbers of fish in replicate ponds. Mortalities ceased after seven days in ponds. The ungraded fish were maintained at high density in order to determine whether density affected growth in the grow out phase, to avoid the mortalities associated with handling the other batches of fish, and because of limitations on number of ponds available.

Fish were fed a commercial sinking barramundi pellet diet (Skretting, Rosny Park, Tasmania) (43% protein, 15% lipid, 22% carbohydrate, 11% ash), with pellet sizes mixed in ratios to suit the size range of fish in each pond. Pellet size ranges were determined by observations of fish gape during sampling and on uneaten food when checking feeding trays. Food consumption was monitored using feed trays and was always to excess. During the grow out phase feed was scattered in about 40% of the pond surface area, at the each end of the pond. After three months feed tray observations indicated that all feeding was taking place at the deep end of the pond; subsequently feeding was limited to that area, over about 25% of pond surface area.

Sampling was done every four weeks commencing 27 days after stocking the ponds. Methods used were the same as those used previously, although pond depth was only reduced by about 40%. Sample size was 52-53 fish in case of deformity or damaged fish. Knotless mesh seine nets of appropriate size were used to sample fish in the morning. Fish collected in the seine were concentrated, then random scoops with a large dip net taken until the required number of fish were collected. Fish were not fed the afternoon before and morning of sampling. Four ponds were sampled daily over consecutive days to reduce differences in sampling and turnaround time before return to the pond. On the last day of the sampling period the remaining three ponds were sampled. All sampled fish were salt bathed for one hour in 10‰ salt before return to the pond.

For the purposes of comparing growth of graded and ungraded fish, the heaviest 50% of the sample from ungraded ponds was counted as ungraded large, and the remainder as ungraded small. Of the sample of fifty fish, the 25 largest fish were treated as ungraded large, and the 25 smallest fish treated as ungraded small. This did permit an error in that only those larger, growing fish were measured as large ungraded, which would add a positive imbalance to the results for that class. However, the splitting of the ungraded fish into small and large did allow estimation of whether there were major differences in growth between graded and ungraded fish.

On the final sampling occasion (295 days after stocking ponds) all fish sampled were sexed. Gentle pressure was applied to the abdomen of anaesthetised fish, if they were males milt was easily expressed. The accuracy of this method was checked by euthanising a sample of 150 fish after they were split into male and female groups by this method, and dissecting them. All male fish had fully developed, ripe testes. Undeveloped gonads were examined using a squash preparation and all were ovarian tissue with developing ova. This was done on three occasions with fish from other ponds but of the same age. This method was 100% accurate (n=150 fish) in determining sex of fish of any size but older than six months. All sexed fish were measured and weighed to determine differences in weight/length ratio.

At the end of the trial, all fish in all remaining ponds were harvested and hand graded into three size classes (<200 g, >200 g and >300 g) for further grow out or sale (Table 6·3). Harvests to complete the trial were staged over a four week period. A random sample of fifty fish from each pond was weighed at harvest, except ungraded treatments in which 100 fish were measured. Ponds of the same treatment were harvested sequentially to minimise the differences within treatments (e.g. all small, graded ponds were harvested on consecutive days, than all large, graded then the ungraded ponds). For presentation purposes, these mean harvest weights are plotted as points on the right hand side of Figure 6·1.

Table 6·3. Percentages of size classes at end of trial. Combined Small plus large (S+L) is small and large graded fish combined for comparison with ratios of ungraded fish.

	% < 200 g	**% > 200 g**	**% > 300 g**
Small Graded	79.22	17.71	3.07
Large Graded	4.04	70.01	25.95
Ungraded	39.87	43.51	16.61
Combined S+L	41.63	43.86	14.51

One ungraded treatment was lost to a *Tetrahymena corlissi* infection, which destroyed most fish before an effective means of control was devised. One pond of large fish was also lost due to operator error while dropping the level to exchange water and reduce pH. The survival rates for these ponds were not included in the final analyses, but their growth rates were included in plots of mean growth per treatment while they were operating.

All comparisons of treatments were done using ANOVA. Prior to analysing growth data, a comparison was made of growth of the prior treatments of point fed and broadcast fed using ANOVA adjusted for covariate, the covariate being the point or broadcast prior treatment. No significant differences were found between these treatments in growth at the end of the trial so the replicates of graded small and graded large fish were pooled to give more replication. At the end of the experiment

there were two ungraded replicates, five graded large and six graded small ponds for comparison.

Growth measured by weight and standard length was compared between the treatments. To compare the variability of the groups ANOVA was performed on the standard deviation of weights or lengths of the replicates. Coefficient of variation which removes the effect of large fish being compared with small fish was also used to compare the amount of variation between treatments, again using weight and standard length as variates.

Differences in sex ratios, sexual dimorphism as measured by weight:length ratio, and proportion of males to females in treatments were all compared using ANOVA performed on weights, lengths, weight length ratio or ration of males to females in the various replicated treatments.

The experiment was approved by the Far North Queensland Animal Ethics Committee, approval number FNQ- 08-01.

6.3 Results

6.3.1 Growth

Growth of fish stocked at differing sizes but the same grade (i.e small or large) was not significantly different. Growth of graded and ungraded groups of fish differed greatly, with ungraded fish lying between the large and small graded fish (Figure 6·1). Growth between all graded groups differed significantly (Table 6·4), the large fish growing faster than the small group over the period of the trial. Size frequency distributions indicated that the small and ungraded groups had high proportions of fish which had limited growth over the period of the trial (74% and 55% less than 120g respectively). Size classes from the grading after harvest (up to one month after the final weigh and measure) suggest that grading had little effect on size frequency, when the graded fish (small and large added together) are compared to the ungraded fish (Table 6·3). Very few small fish grew to exceed 300g. Variation in growth (CV)

was not significantly different between the graded treatments, but was high anyway at 62 and 71% for small and large groups respectively (Table 6·4).

Specific growth rate over the course of this trial was highly variable but generally followed a similar pattern of stable growth rate after an initial high rate just after stocking. There was a slight decline in SGR over the course of the trial as the fish grew (Figure 6·2).

Table 6·4. Weight and length of fish (means ± SE, standard deviation and coefficient of variation) of at the end of the grading experiment. Different superscripts indicate significant differences at P< 0.001.

Variate	Mean of small graded	Mean of ungraded	Mean of large graded	Mean LSD
Weight	107·6 ± 10.83	165·7 ± 22.43	235·1 ± 20.56	
SD of weight	76·6 [a]	158·6 [b]	145·4 [b]	31.23
CV weight	0·7015 [a]	0·9635 [b]	0·6191 [a]	0·1507
Length (mm)	149·0 ± 4.09	166·3 ± 6.09	191·3 ± 4.83	
SD of length	28·93 [a]	43·07 [b]	34·18 [a]	7·825
CV SL	0·1930 [a]	0·2589 [b]	0·1792 [a]	0·04362

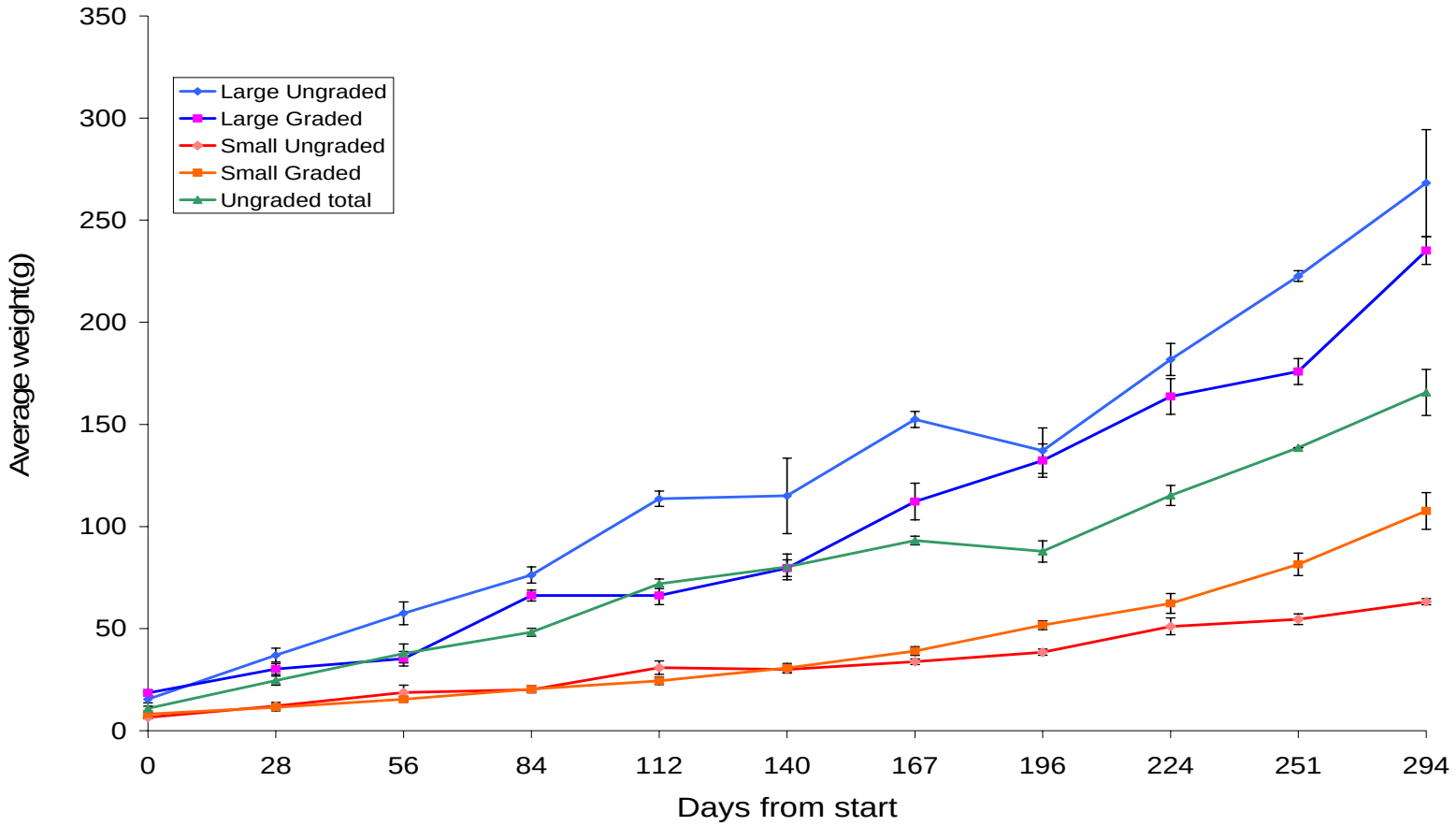

Figure 6·1 Mean (±SE) weight of graded and ungraded golden perch. Large ungraded and small ungraded are subsamples of the samples from ungraded ponds. Large graded and small graded are the respective graded fish.

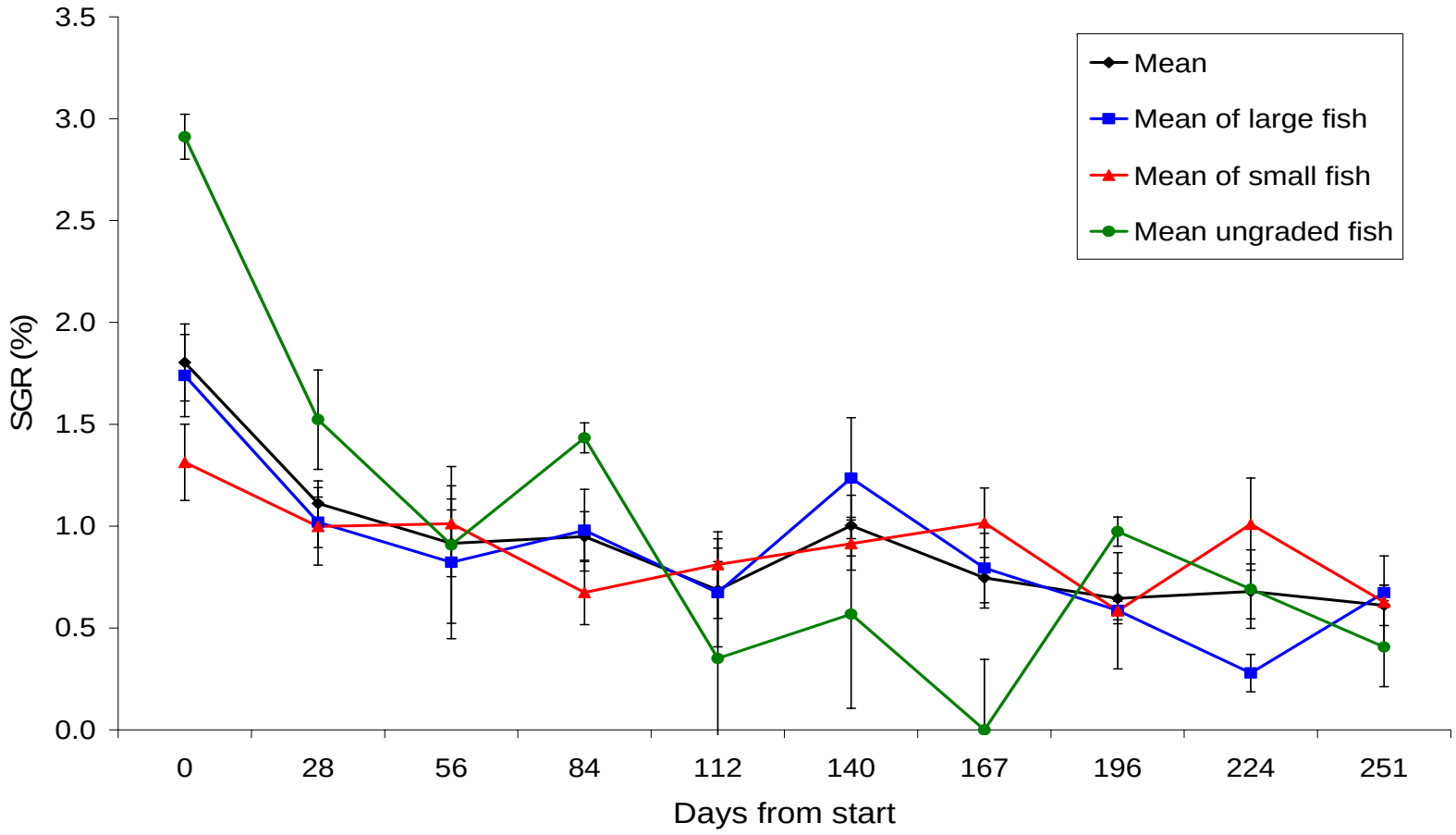

Figure 6·2 Mean (± SE) specific growth rate (percentage growth per day) of graded and ungraded golden perch over 35 weeks from April to December 2002. Means are of all fish sampled.

6.3.2 Survival

Grading and handling resulted in significant mortalities of juvenile golden perch, despite use of sedation and salt (10‰) to reduce stress during handling. Mortality of fish of 10-37% (mean 26.8%) in small graded fish, and 6-37% (23.4%) in large graded fish in the week after handling these fish indicated that grading was probably the causative factor, as routine sampling of fish never resulted in mortalities. Otherwise, survival was very high in all treatments.

Table 6·5. Survival of fish in ponds. All mortalities occurred immediately after grading. Mortalities due to operator error (B2) and tetrahymenosis (B6) are not included. Sg = small graded, Ug = ungraded and Lg = large graded treatments.

Pond	Treatment	N stocked	N harvested	% survival
B1	Sg	569	336	59.1
B2	Sg	560	376	67.1
B3	Ug	1266	1238	97.8
C2	Lg	592	453	76.5
C3	Lg	579	449	77.5
C4	Ug	1265	1190	94.1
C5	Lg	579	555	95.9
C6	Lg	574	364	63.4
D1	Sg	560	426	76.1
D2	Sg	578	437	75.6
D3	Sg	546	470	86.1
D4	Lg	560	269	48.0
D5	Sg	537	443	82.5
D6	Lg	549	481	87.6

6.3.3 Sex ratio

There was no significant difference in the proportion of males between treatments, although the proportion of males in the ungraded treatment was lower than either of the graded treatments (Table 6·6). The size frequency distribution of pooled results indicated a strongly bimodal frequency distribution for females (Figures 6·3 – 6·6) with 100 % of fish below 15 g being female and another peak of females in the higher weight ranges (>500 g). However, this was not reflected in the distribution of graded fish. Males were proportionally dominant in the middle of the size distributions (50-500 g). Pooled results of sex ratio indicated a much larger proportion of males in the population than females, with 64% males and 36% females.

Table 6·6. Means (±SE) of variables measured in males and females in graded and ungraded treatments of pond grown golden perch.

Variate	Mean of small graded	Mean of ungraded	Mean of large graded
Weight male	112·1± 5.12	156·7 ± 13.39	221·1 ± 9.66
Weight female	96·1 ± 10.14	187·2 ± 35.59	263·0 ± 20.38
Length male	152·2 ±1.92	168·7 ± 4.58	189·8 ± 2.35
Length female	141·4 ± 7.98	163·9 ± 8.60	194·0 ± 4.72
Wt/SL ratio male	0·6892	0·8455	1·1154
Wt/SL ratio female	0·5937	0·9110	1·2340
Proportion of males	70·7%	62·0%	69·2%

The smallest fish sexed were 9 g female and a 10 g male. The male had fully developed ripe testes and expressed milt easily; the female had developing ova in the ovaries discernable with the naked eye. All male fish dissected had testes white and broad, extending forward to the head kidney. Both sexes, when spent or out of season have thin, barely discernable tubes extending from the cloaca anteriorly. Sexual dimorphism was marked, with females becoming heavier than males after reaching about 150mm SL. There were differences in weight length ratios for both males and females between the two treatments (Table 6·6), suggesting a change in body shape at around this size.

There is a significant exponential relationship between weight and standard length ($P<0.001$). The equations for male and female are also significantly different (significantly different non-linear parameters $P<0.01$). The curves for male and female start to diverge between 150 and 200 mm SL (Figure 6·3).

The general equation is:

$$Weight = A + B*(R^{SL})$$

$$Female: Weight = -59.20 + 21.37* (1.013316^{SL})$$

$$Male: Weight = -71.15 + 27.12*(1.012106^{SL})$$

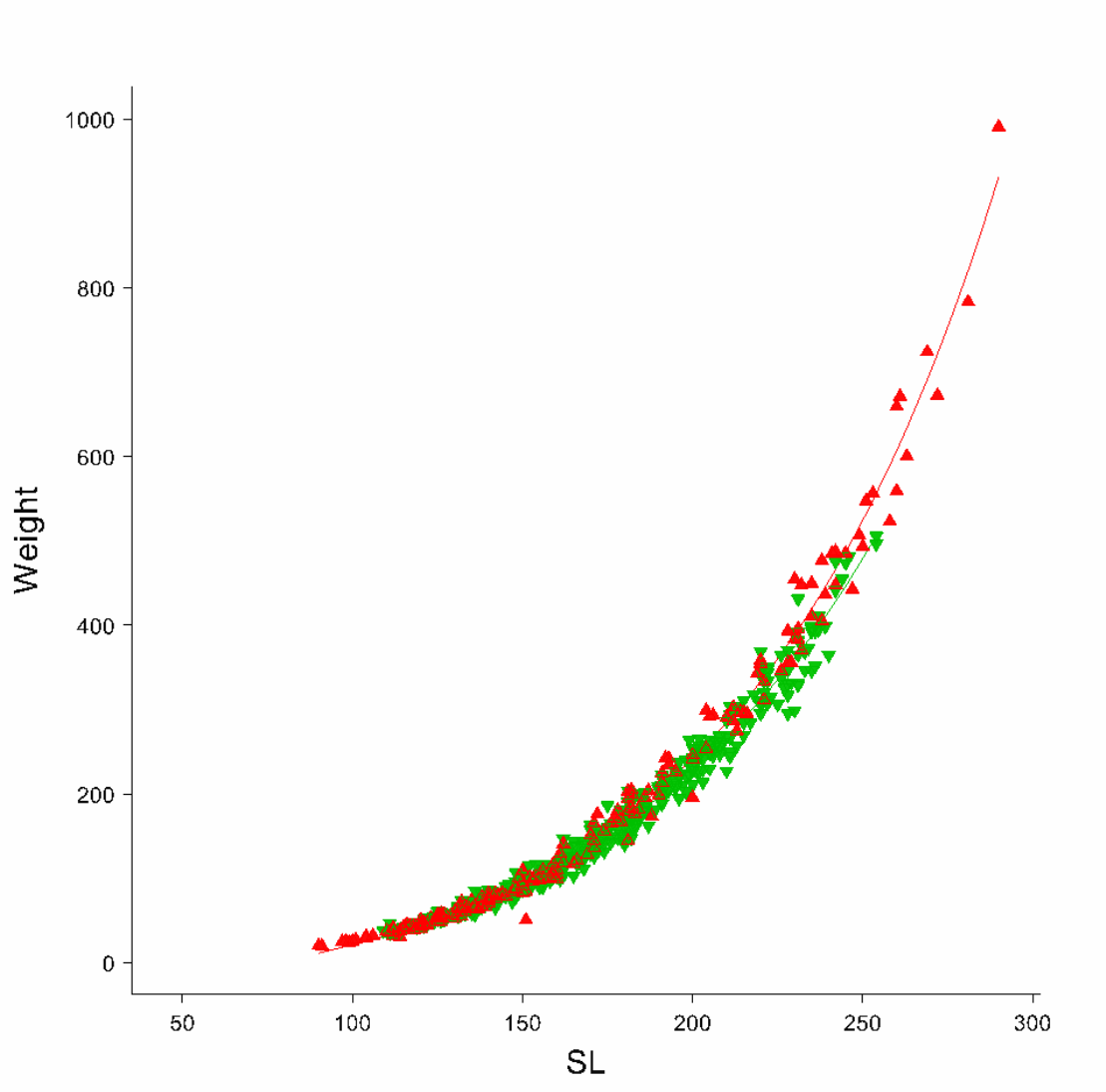

Figure 6·3. Fitted (lines) and observed relationship of weight against standard length for male (green) and female (red) golden perch from growout trials. Total n = 493 male, 255 female.

Table 6·7. The effect of prior treatment history on mean (±SE) growth of golden perch. The sex ratio is number of males per female.

Prior treatment and grade	Sex Ratio	n (ponds)	Mean (±SE) weight	
			F	M
Broadcast small	2.28	3	107.97 ± 18.19	124.15 ± 34.7
Broadcast large	2.23	2	263.61 ± 0.1	211.28 ± 18.92
Point small	2.58	3	84.24 ± 0.15	100.10 ± 15.64
Point large	2.46	3	262.53 ± 12.73	227.65 ± 19.39
Ungraded	1.36	2	187.24 ± 8.86	156.68 ± 23.79

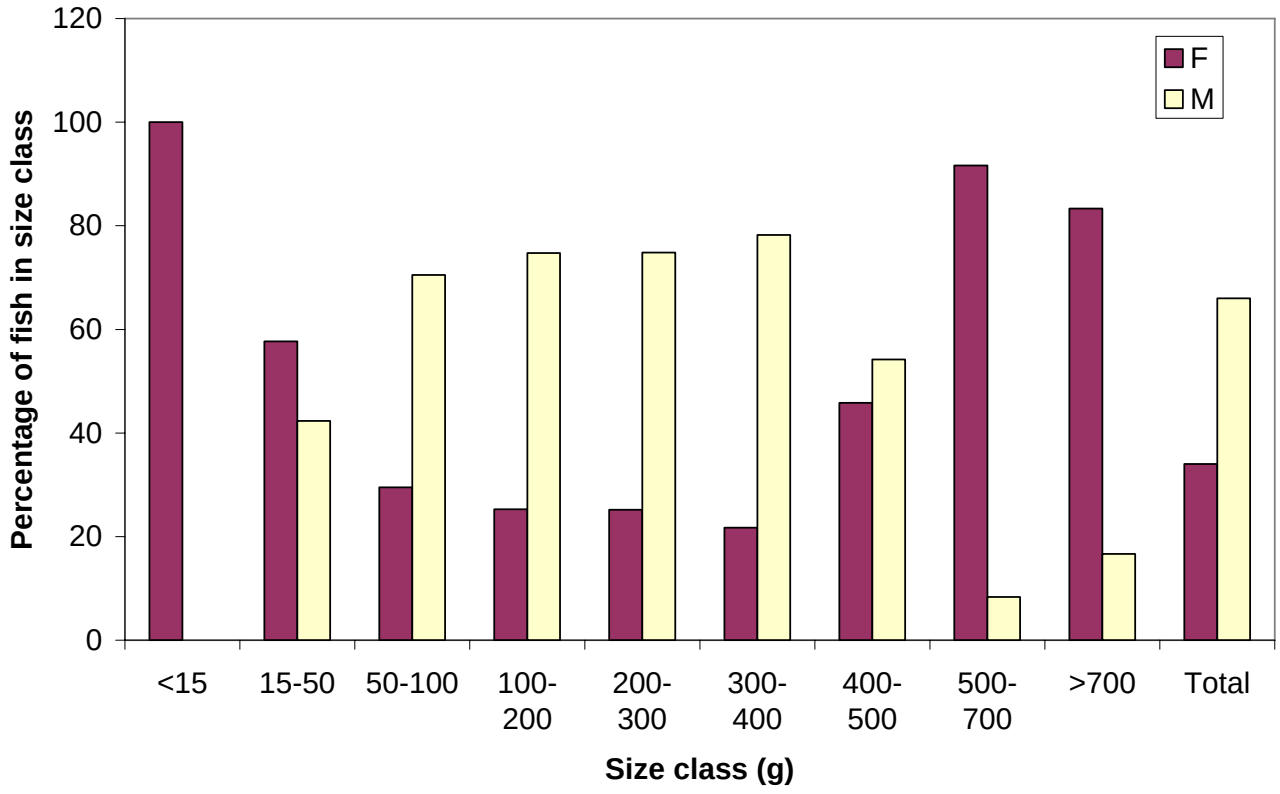

Figure 6.4. Size frequency histogram of golden perch - expressed as a percentage of each sex in each size class.

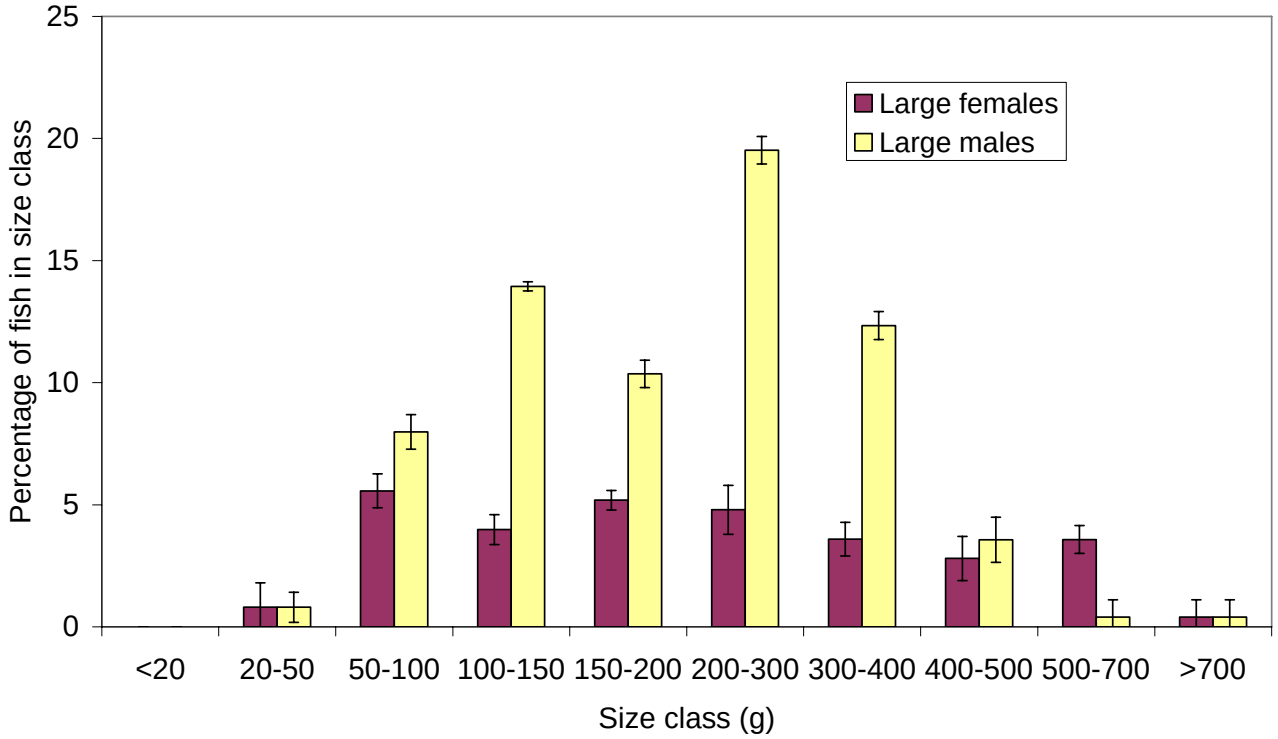

Figure 6·5. Size frequency distribution of large, graded golden perch after nine months growout. Expressed as percentage of total population.

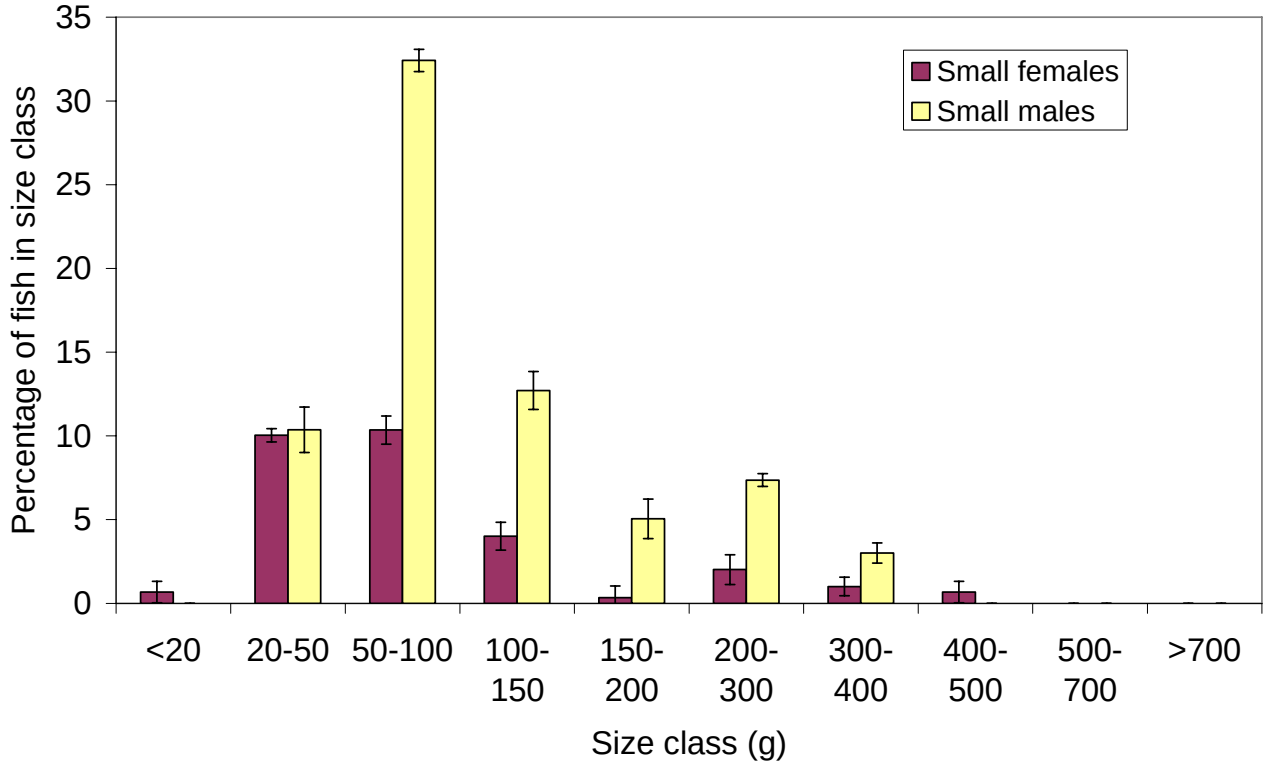

Figure 6·6. Size frequency histogram of small, graded male and female golden perch after nine months growout. Expressed as percentage of total population.

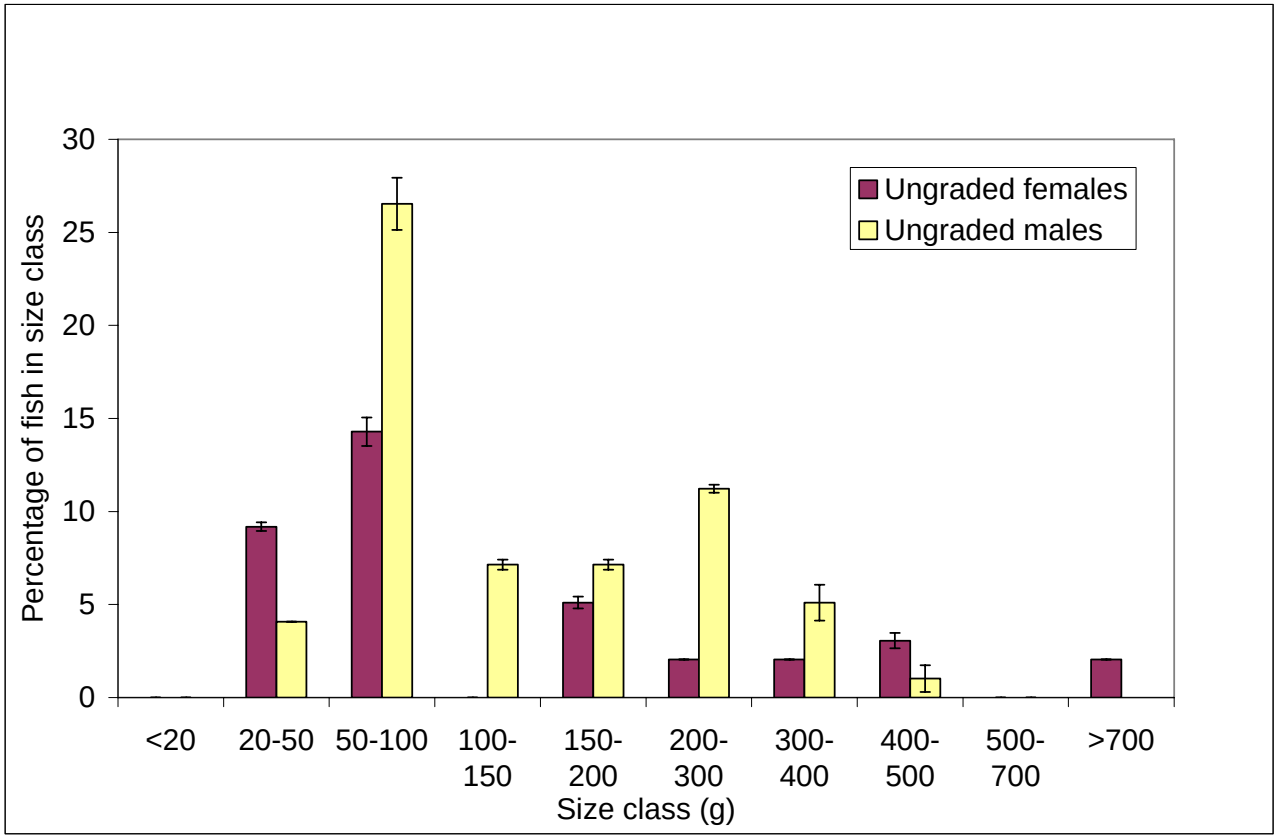

Figure 6·7. Size frequency histogram of ungraded male and female golden perch after nine months growout. Expressed as percentage of total population.

6.4 Discussion

Growth was strongly linked to grading and the results of this study indicate that, in the short term, the bottom 50% of golden perch do not grow rapidly enough to justify the operational expenses of keeping them. Females in the small graded class were on average smaller than males in this treatment. It appears that some female golden perch do not grow for some unknown reason, negatively biasing the mean growth rates of females. If the triggers that reduce growth potential could be identified, it could address this issue. In some other species, starved fish also have good growth potential which is expressed when external inhibitory factors are removed. In grouper (*Epinephalus coioides*) presence of large fish restricted availability of food, and when large fish were removed the stunted ones grew (Bombeo-Tuburan *et al.*, 2002). In redclaw (*Cherax quadricarinatus*), removal of social inhibition of food availability permitted rapid growth of 'starved fish' (Barki and Karplus, 2004). Growth of golden perch is comparable to some other high value species such as grouper (e.g.

Epinephelus coioides grew to 238g in 8 months (Bombeo-Tuburan *et al.*, 2002), but not to fast growing fish such as silver perch and barramundi. There was no expression of fast growth potential in small golden perch after grading, during the period of the trial, suggesting that in golden perch social inhibition is not the prime factor influencing growth of smaller fish.

The lower number of females relative to males in the graded treatments suggests that the mortalities experienced after grading and stocking the ponds were dominated by females, or that effects of feeding during the nursery phase influenced sex determination of at least some individuals. Although environmentally determined sex differentiation is a well known phenomenon and is influenced by density, nutrition and behavioural factors (Strussmann *et al.*, 1996; Stewart *et al.*, 2001; Devlin and Nagahama, 2002), in this case the ungraded fish had a substantially lower ratio suggesting that grading produced sex-specific mortality. In contrast to these findings, sex ratios of European seabass are not influenced by grading during their sex-labile period (Saillant *et al.*, 2003). Grading of channel catfish becomes sex selective at an early age due to differing morphological characteristics (Goudie *et al.*, 1993). It is suggested that grading of golden perch *per se* had no effect on sex ratio, but that mortality afterwards was sex selective and selected for faster growing female fish. The morphological differences appeared to be size dependent, and appeared at about 150mm SL in female fish.

Table 6·8. Predicted weights of golden perch of Battaglene (1991)(B) and the current study (cs).

TL (mm)	SL (mm)	Weight M(cs)	Weight M(B)	Weight F(cs)	Weight F(B)
150	128	55.39	88.36	56.99	55.36
200	175	151.61	184.02	157.15	133.89
250	207	256.24	325.08	271.17	265.62
300	242	427.71	517.49	465.69	464.88
350	285	765.80	766.69	867.85	746.23

Because females tend to be heavier at a given length than males, grading and subsequent sex selection by mortality need to be managed in a production situation. The difference in weights of sexes between the small and large graded populations reflects the bimodal distribution of females. The small graded females were smaller than their counterpart males, but the large graded females were substantially larger than the males. Although ungraded females were larger on average than the males, selective mortality may have primarily affected the faster growing fraction of the female fish in graded treatments. This definitely indicates a requirement for development of a better handling technique during grading to eliminate or reduce grading related mortality. Interestingly, preliminary trials on growout of Lake Eyre golden perch ran into similar problems, except with almost compete mortality of all fingerlings during grading on three separate farms in 2004. Reports from silver perch growers also suggest that a small degree of mortality after handling, indicating that research into handling of Australian native fish in pond culture is needed to eliminate this problem.

The lack of a difference in sex ratio between small and large fish is an indication that the differential growth between males and females does not start prior to grading. Battaglene (1991) and this study supports the notion that sex influenced growth and morphology only start when fish are older than 3-4 months. As all male fish had fully developed testes regardless of size when examined (the smallest fish examined were 9 and 10 g), it appears that sex and maturity in golden perch may be age determined independent of size. Whether fish could breed at such small sizes is immaterial- diversion of energy to gonad development with potential concomitant negative impact on growth is not desirable in aquaculture.

The sex ratios in this study (62-70% males) correspond to those of Battaglene (1991) (71% males). In Battaglene's study this imbalance was attributed to shoaling behaviour of males. The results of the current study suggest that higher ratios of males may be normal in both wild and natural populations. However, given the variability of sex ratios from different parentages the results of this study should be treated with caution. The projected weights from lengths suggest that cultured and wild female golden perch are very similar in length/weight ratio but that males differ from a smaller size.

6.5 Conclusion

The results of these experiments demonstrate that a high density nursery phase could be used prior to grading and redistributing fish. Broadcast feeding of ponds increases retention of fingerlings on formulated food and initial growth rates, but resulted in little difference in ultimate growth rates of the graded fish. Improvements in retention rate could probably be made by reducing access to zooplankton. Low productivity turbid water may assist in the process. Soils in inland Queensland have a kaolin clay content which contributes to high turbidity and low natural productivity, so it is likely that golden perch culture in these areas could be a viable option for multiple water use. This is in direct contrast to many other fish species where natural productivity is encouraged.

The densities at which golden perch grow well indicate potential for intensive culture. Lower densities may not produce better growth as growth appears to be largely driven by diet and genetic factors. Behavioural influences as tested in these experiments did not appear to affect growth in the 7 months of growout after grading. However, this result is not conclusive as selective mortality of faster growing females in graded treatments biased results. The faster overall growth of females and rapid separation of growers and non-growers after the three month periods suggests that all female culture would be an option worth investigating. The proportion of females that do not grow at all would be offset by the rapid growth of the others. Also, the small fish would incur fewer costs in terms of oxygen, food and ammonia production.

Development of a food for golden perch, and better handling techniques, could substantially increase productivity and reduce FCR. The likely ability of golden perch to be cultured in turbid water environments increases their potential for farming in inland Australia.

Chapter 7
Tetrahymenosis, Columnaris disease and motile aeromonad septicaemia in golden perch, from Australia.

7.1 Introduction

Parasitic, fungal and bacterial diseases of golden perch have been well documented (Rowland and Ingram, 1991). However, to date, there has been no report of *Tetrahymena sp*. infestation in this species. *Tetrahymena* spp. are ubiquitous free-living hymenostome ciliates, that cause disease in a wide variety of fish, crustaceans, amphibians and turbellarians (Hoffman *et al.*, 1975; Wright, 1981; Lom, 1984; Ferguson *et al.*, 1987; Lom and Dyková, 1992; Edgerton *et al.*, 1996; Ponpornpisit *et al.*, 2000). Species pathogenic to fish include *T. corlissi*, *T. faurei, T. pyriformis,* and *T. rostrata. Tetrahymena corlissi* has been recorded as a parasite of freshwater tropical fish and amphibians, is histophagous, and can destroy surface tissue, invading the skin, skeletal muscle and internal organs, causing mass mortalities in guppies *Poecilia reticulata* and dwarf gouramis *Cloisa lalia* (Lom and Dyková, 1992; Imai *et al.*, 2000; Wakita *et al.*, 2002). *T. corlissi* has been associated with gross clinical signs of epidermal sloughing, raised scales, skin ulceration and lesions, and skeletal muscle inflammation and necrosis. Clinical disease is rapid in onset and often fatal (Lom and Dyková, 1992; Imai *et al.*, 2000; Wakita *et al.*, 2002).

Aermonads are motile Gram negative bacteria, ubiquitous in aquatic ecosystems. These bacteria are routinely isolated as both normal flora and as primary or secondary pathogens from sick or moribund fish. Motile aeromonad septicaemia may cause acute or chronic infections in many different species of freshwater fish. The bacteria responsible (*Aeromonas caviae*, *A. hydrophila* and *A. sobria*) all produce extracellular enzymes and toxins causing cell lysis and necrosis (Roberts, 1993). Acute infections often manifest with clinical signs of anorexia, exophthalmia, abdominal distension and septicaemia, whereas chronic infections are characterised by deep dermal ulcers, haemorrhage and inflammation. The disease is strongly correlated with stress, overcrowding, poor hygiene and stress-mediated immunosuppression (Toranzo *et al.*,

1987; Roberts, 1993; Thune *et al.*, 1993). Previous motile aeromonad septicaemia (caused by *Aer. sobria*) of golden perch at FFAC had symptoms of petechial haemorrhage on the ventral surfaces of the fish, disoriented swimming behaviour, and septicaemia leading to death in 4-7 days (unpublished observations).

Columnaris disease caused by *Flavobacterium columnare* has been documented worldwide in over 36 species of freshwater fishes including the cultured species, barramundi *Lates calcarifer*, channel catfish *Ictalurus punctatus*, tilapia *Oreochromis* sp. and ornamental fish including goldfish *Carassius auratus* (Carson *et al.*, 1993; Soltani *et al.*, 1996; Plumb, 1999). Gross lesions typically include gill necrosis, skin ulceration, jaw erosion and fin and tail rot to varying degrees. Columnaris disease at FFAC is usually manifested as a saddleback lesion across the body, usually posterior to the second dorsal fin (Mosig, 2002). Outbreaks of columnaris disease are associated with environmental factors including low or high water temperatures, low salinity, crowding, high organic loads, handling, poor nutrition and stress (Chowdhury and Wakabayashi, 1991; Wakabayashi, 1991; Carson *et al.*, 1993; Soltani and Burke, 1994; Altinok and Grizzle, 2001; Shoemaker *et al.*, 2003).

In two successive winters of 2001 and 2002, golden perch grow out trials at FFAC were devastated from mass mortalities of fish from infections with *Tetrahymena*, motile aeromonad septicaemia and columnaris disease . This paper describes the gross, histology, microbiology and parasitology findings from golden perch affected by these diseases. Results from chemotherapeutics used in ponds and *in vitro* trials in tanks against *Tetrahymena corlissi* are also presented.

7.2 Methods and Materials

7.2.1 Grow out environment and subject clinical history

Grow out trials were conducted on golden perch fish, in 2000/2001 and 2001/2002. Two groups (A and B) were grown in 2000/2001, and one group (C) in 2001/2002. Facilities used in the trial are described in Chapter 5 (section 5.2.1) and fish treatment collection in 5.2.2. Aspirators provided supplementary aeration and mixing, as required during pond chemotherapy treatments. Pond preparation prior to stocking

included removal of organic matter, liming with agricultural lime at 1.2 tonnes/ha and dry-out for one month. Incoming water was filtered with 500 μm screens. Water quality parameters in ponds and in tanks measured daily included pH, pond water temperature, and dissolved oxygen. All dead fish and sick fish were removed from ponds daily and recorded. All fish were fed daily to satiation with a sinking barramundi grower diet (Nutreco Pty Ltd, Rosny park, Tasmania) (43% protein, 15% lipid, 22% carbohydrate, 11% ash). Feed trays were used to monitor feed consumption.

Group A: In March 2000 two different strains of fingerling golden perch (Murray-Darling strain and Fitzroy River strain) (30-50 mm TL) were purchased from a commercial hatchery. The two strains were weaned in tanks before transfer to four ponds in May. Water lettuce (*Pistia stratiotes*) was introduced into the ponds from local ponds in December 2000 for algal control and to provide fish with shelter. In April 2001, fish were graded and moved into 4 new ponds (Table 7·1). Fish were again graded in April 2001 and moved into newly prepared ponds, along with the water lettuce. Water was sourced from a ground water well.

Table 7·1. Mean (± SE) weights for group A fish.

Pond	Strain	Size class	Mean weight	Number of fish (per m^2)	Approximate biomass (kg/m^3)
B2	Murray-Darling	Large	357.8 ± 27.99	289 (1.26)	0.323
B3	Murray-Darling	Small	61.98 ± 2.58	289 (1.26)	0.056
B4	Fitzroy	Large	342.5 ± 16.65	302 (1.31)	0.323
B5	Fitzroy	Small	128.7 ± 8.26	302 (1.31)	0.122

Group B: Fingerling golden perch arrived at Walkamin on 24 Nov 2000, were weaned and stocked into nursery ponds on 22 Dec 2000. These were harvested, graded and stocked into new ponds on 14 May 2001. During grading fish were treated with a salt bath (12‰) salt for 60 to 90 minutes. They were then sedated, caught and transported back to new ponds in 10‰ salt water, on 15-17 May (Table 7·2). Fish from all ponds were mixed for statistical purposes, to have a complete mix of fish from experiment 5.2.

Table 7·2. Mean (±SE) weights and size classes of group B fish.

Number of ponds	Size class	Mean weight	Number of fish (per m^2)	Approximate biomass (kg/m^3)
6	Small	32.28 ± 0.82	582-676 (1.82-2.11)	0.082 – 0.095/m^3
3	Medium	113.21 ± 1.37	457-498 (1.43-1.56)	0.23-0.24/m^3
1	Large (Top 2%)	197.19 ± 4.01	141 (0.44)	0.12/m^3

Group C: Fingerling *Macquaria ambigua* arrived at Walkamin on 1 Nov 2001, were weaned, and 1,266 were stocked into each of 6 ponds on 19 Dec 2001. These ponds had been treated with 20 mg/L chlorine and sun dried for 6 months. Fish were sampled once every four weeks, and there was no grading. These ponds were treated with Platypus Probiotic (International Animal Health products Pty. Ltd., Huntingwood, Australia), a program of weekly treatments administered over a four-week cycle to introduce large numbers of beneficial *Bacillus sp*. bacteria into the water. The probiotics were administered according to the manufacturer's instructions.

7.2.2 Gross pathology, histology and microbiology

From the outset and throughout all infections gross observations and fresh scrapes and smears were taken from sick and undamaged fish at regular intervals (at least weekly) to assess progress of infections, check efficacy of therapeutic treatments, and to ensure that the same infection was present throughout the course of the epizootic. In May 2001, January and July 2002 samples of moribund golden perch from these epizootics were submitted to Oonoonba Veterinary Laboratory (OVL), for gross, histological, parasitological and microscopic examination. In July 2002, further samples of golden perch with 3 differing types of skin lesion were submitted for histological examination to determine the aetiology of each type of lesion.

In June 2001, fish with grossly visible small red 'pinpoint' skin lesions, 1 to 2 mm diameter, were observed to monitor progression of the skin lesions over time.

Fish were sacrificed, organs and tissues including the various skin lesions gills, brain, heart, liver, head and caudal kidney, spleen, pancreas, eye, stomach, intestine, abdominal fat, swim bladder, bladder, skin and skeletal muscle were fixed in Bouin's fixative for 24 hours then processed routinely for histology (Bancroft and Stevens, 1990).

Movement and morphological characteristics of live protozoan parasites observed on wet mount preparations were studied for species identification at both FFAC and OVL. Air dried skin smears were either stained with Klein's dry silver impregnation method (Lom and Dyková, 1992) or preserved in Bouin's fixative, and sent to Dr. Peter O'Donoghue (University of Queensland Department of Parasitology) for species confirmation. Skin scrapes were taken from the leading edge and from deep within the centre of skin ulcers and plated onto Sabourads dextrose agar (SDA) with added chloramphenicol and gentamycin, and marine agar with added thiamine (MAT) for fungal isolation and culture. Swabs were taken from the skin ulcers, heart and caudal kidney of several fish with skin ulcers, and plated onto sheep blood agar (SBA) and marine agar with added vitamins (MAV) for bacterial isolation and culture.

7.2.3 Pond treatments and chemotherapeutic tests

Chemicals recommended by Boyd (1982), Kabata (1985) and Noga (1996) were added to ponds containing affected fish for the treatment of the *T. corlissi* infection. Four ponds were treated with 30 mg/L formalin, then 8 days later with 7 mg/L potassium permanganate ($KMnO_4$), then 3 days later $KMnO_4$ at 5 mg/L. $KMnO_4$ demand was calculated according to Boyd (1982). Two ponds were treated with 20 mL/L formalin, four days later formalin at 30 mL/L, the five days later 7 mg/L $KMnO_4$, the 3 days later 5 mg/L $KMnO_4$. Samples of sick fish were taken approximately six hours and 24 hours after each treatment from each pond and wet smears from scrapes examined. Formalin was added to 2 ponds at 15 and 30 mg/l. Prior to chemotherapy application the water level was dropped in ponds, and additional aspirators were placed in the pond to increase mixing and dissolved oxygen levels. Each chemical was added to the inflow water to ensure distribution and mixing of the chemical in the pond water.

Chemotherapeutics recommended by Boyd (1982), Kabata (1985) and Noga (1996) for the treatment of ectoparasites were tested on infected fish from the 2001 outbreak as small volume treatments (Table 7·3). For each chemical tested, 5 small (<50 g) fish with skin ulcers were placed in an aerated 20 L plastic bucket containing the chemical. At the end of each chemical treatment, skin scrapes were taken from the edges and at the centre of skin ulcers of each fish, wet mounts were prepared and observed for the presence and activity of *Tetrahymena sp.* under a light microscope.

Table 7·3. Chemotherapeutic trials. As all fish had severe lesions, not all survived for the full length of the trial. At least two fish from every trial were alive at the end.

Chemical	Concentrations	Duration	Method
$KMnO_4$	1, 5, or 15 mg/L	1 and 20 hours	20 L bucket
Malachite green	0.1 or 1 mg/L	1 and 20 hours	20 L bucket
Methylene blue and salt	1 or 3 mg/L MB 10‰ salt	1 and 20 hours	20 L bucket
Formalin and salt	70 mg/l formalin salt (12‰)	1 and 5 hours	20 L bucket
$CuSO_4$	25 mg/l	1 hour.	20 L bucket
Salt	10‰	5 to 7 days	100 L tank

In June 2002, fish in the pond affected with tetrahymenosis were fed with Emtryl Soluble (400 g /kg dimetridazole soluble powder, Rhône-Poulenc/ Aventis Animal Nutrition Pty Ltd). Fish were administered the drug orally at a dose rate of 30 mg dimetridazole /kg/fish for 10 days. Emytryl was dissolved and sprayed onto the food with gelatine. Gelatine was used to help the drug stick to the pellets and increase retention time on pellets while in the water. Feed consumption was monitored daily using feed trays. Several fish affected with tetrahymenosis were taken out of ponds and placed in 200L tanks and similarly administered with Emtryl for observation to assess healing of skin ulcers and health in response to administration of the antiprotozoal. Skin scrapings were then done from the edge and centre of skin ulcers to determine whether *T. corlissi* was still present.

In 2001, due to continuing deaths of fish with no apparent reduction, attempts at disease management were suspended and all remaining fish euthanised in late June. All ponds were scraped clean, chlorinated then sun dried for six months prior to restocking with fish.

7.3 Results

7.3.1 Growout mortality and clinical history

The first mortalities occurred in April 2001, one day before fish were handled, graded and transported to new ponds. Mortality rates of affected fish in the 14 ponds in 2001 varied from 32% to 86%, with an average of 40% ± 18.7%. In the winter of 2002 mortalities was 92% in the single pond affected. In both years, mass mortalities coincided with cool water temperatures (Figures 7·1 and 7·2).

Water quality parameters were within normal range for ponds at FFAC. pH varied from 7 to 9 during the entire period of the growth trials. Dissolved oxygen was maintained at above 90% saturation. Pond water temperature varied during 2001 from a minimum of 17.6^{o}C in winter to a maximum of 26.6^{o}C in summer (Figure 7·1 for winter temperatures). In both winters a drop in pond water temperatures of 3.5^{o} C in 7 days was noted (mid-May and mid-June in 2001 and 2002 respectively).

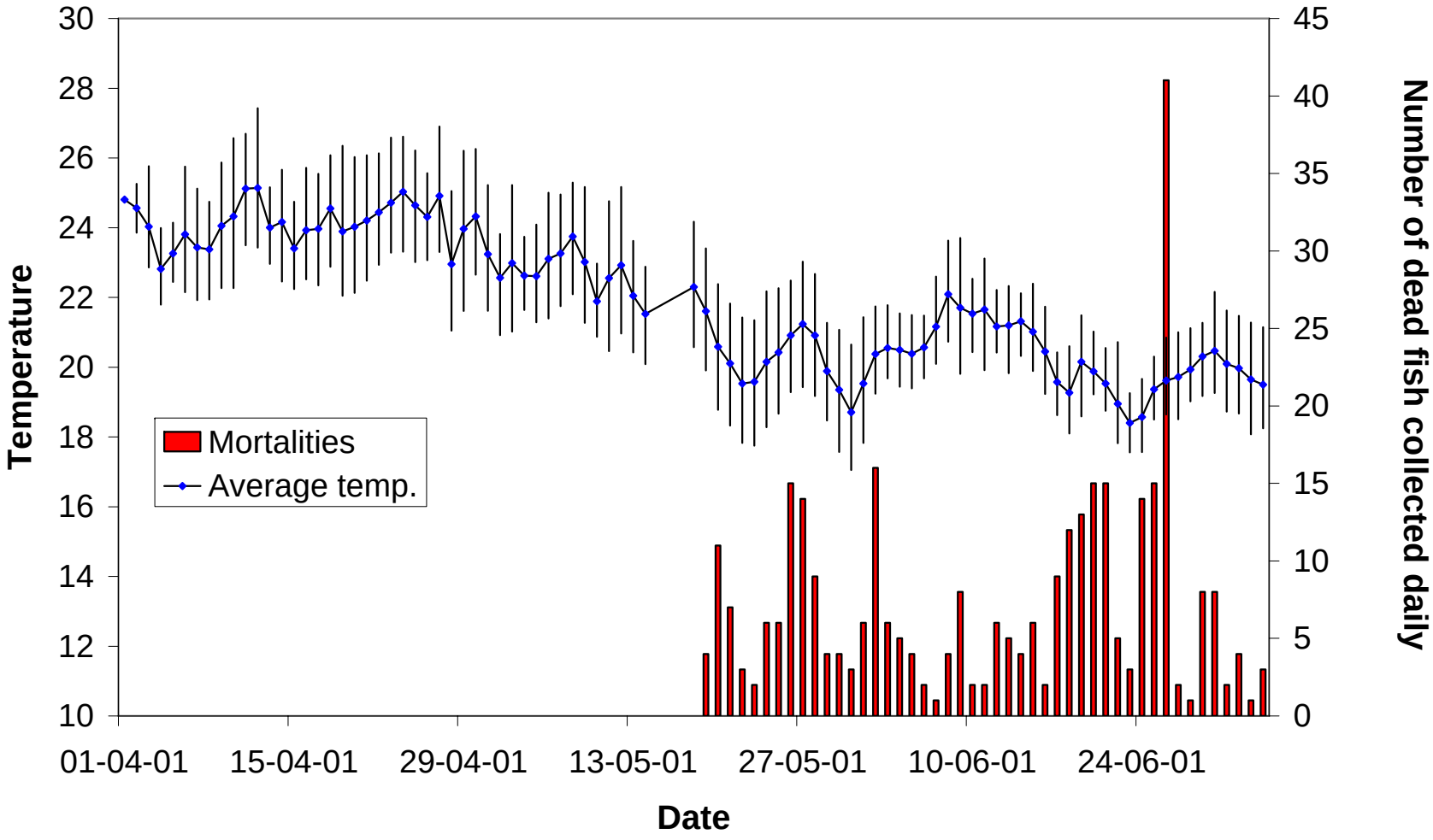

Figure 7·1 Mortalities caused by Tetrahymena in a typical pond, and temperature, in the 2001 epizootic (Group B).

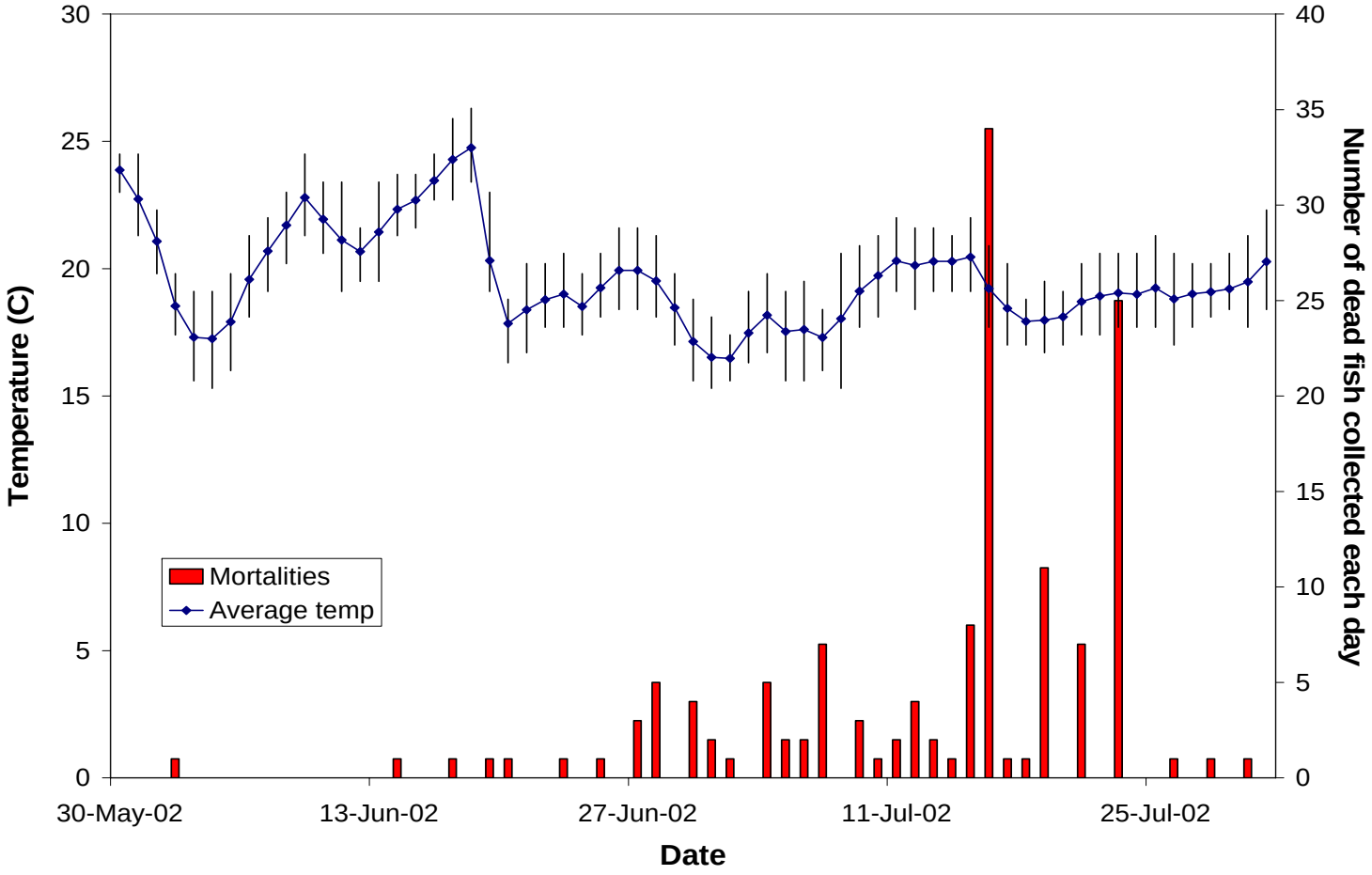

Figure 7·2 Mortalities in the single pond affected in 2002 (Group C).

7.3.2 Gross pathology, histology and microbiology

Fish submitted in May 2001 (groups A and B) showed reddening of the pectoral, dorsal and anal fins, and ulceration of the skin with ‘saddleback’ lesions under the dorsal fins typical of infection with columnaris disease caused by *Flavobacterium* sp. The gills of all infected fish were anaemic. Fish had large, round deep skin ulcers with hyperaemic margins exposing the skeletal muscle (Figures 7.3 - 7·7). The skin ulcers were located variously on the body of the fish but were most common laterally or the caudal peduncle. Several fish had white, fuzzy growths attached to the skin ulcers, resembling that of fungal infection. Gross internal examination of several infected fish with deep skin ulcers showed many had peritonitis with a red-brown exudate typical of motile aeromonad septicaemia.

Figure 7·3. Location of lesion on the side of the fish.

Figure 7·4. Early lesion on side of a golden perch. Note deep lesion into skeletal muscle and early haemorrhage around margins. There appears to be almost no immune response (oedema, erythraemia) at this point.

Figure 7·5. Early lesion with haemorrhagic margin on one side. Scales in middle of lesion are lifting.

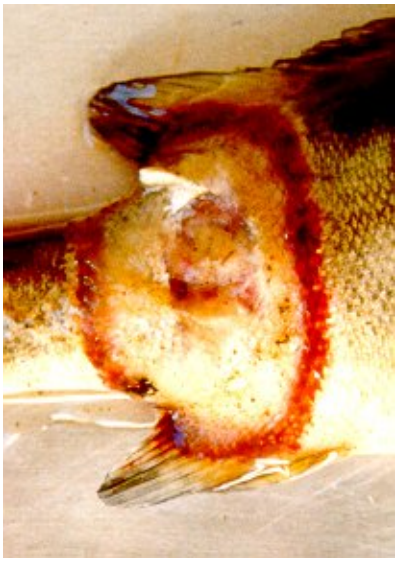

Figure 7·7. Advanced lesion. Haemorrhagic margin of lesion with skeletal muscle necrosis and secondary infection with fungi and bacteria. Extremely high numbers of *Tetrahymena* were found at the margins of such lesions, and fewer throughout the necrotic and dead tissue.

Fish submitted in July 2002 (Group C) had different types of grossly visible skin lesions; including small 1-2 mm dark marks on intact skin on the caudal peduncle, deep round skin ulcers with haemorrhagic margins varying in size from 0.5 to 5 cm, areas of shallow skin erosion beneath the dorsal fin with reddening of the caudal fins, and areas of skin that were pale in colour but with no erosion, rostral to the dorsal fin.

Observations made on ulcer development on fish held in tanks in 2001 (group B) with visible small 'pinpoint' red skin lesions, 1 to 2 mm diameter, showed the lesions grew rapidly into skin ulcers that reached 5 to 7 cm diameter within 2 to 4 days, occupying up to 10% surface area of the fish before death of the host. Small fish (30-120 g) died within 1-2 days whereas larger fish (>120 g) died within 2 to 5 days.

Examination of wet mount preparations of skin smears from the leading edge of areas of skin ulceration in fish with "saddleback' lesions under the dorsal fins in May 2001 showed numerous filamentous, gliding bacteria forming 'haystacks' typical of *Flavobacterium columnare*. Wet mount preparations also revealed thousands of active hymenostome ciliated protozoan parasites, pyriform in shape with somatic cilia

covering their entire surface. Scrapes prepared from the centre of the skin ulcers had fewer parasites, and presence of fungal hyphae. Skin scrapes from normal areas of skin were negative for ectoparasites. Examination of wet mount preparations from gill smears showed low numbers of the flagellated protozoan parasite *Ichthyobodo necator*, the ciliated protozoan parasite, *Trichodina* sp. and *Ichthyophthirius multifiliis.*

Wet mount preparations sent to Queensland University Microbiology Department for confirmation of identification and identified as *Tetrahymena corlissi* Thompson, 1955 on the basis of their characteristic morphological features (Table 7.4).

Table 7.4 Morphometric characterization of *Tetrahymena corlissi* (X = mean, SE = standard error, min = minimum, max = maximum, n = number of observations). Provided by Peter O'Donoghue, University of Queensland. All length measurements in μm.

THIS TABLE HAS BEEN REMOVED DUE TO COPYRIGHT RESTRICTIONS

7.3.3

Histological examination of deep skin ulcers from fish submitted in May 2001 (Groups A and B) and July 2002 (Group C) showed the skin was ulcerated to the level of the skeletal muscle, and occasionally to the abdominal cavity. Colonies of Gram-negative bacilli were seen among necrotic dermis and necrotic skeletal muscle. Numerous *T. corlissi* were detected in the scale pockets, epidermis, dermis and

between necrotic bundles of skeletal muscle. In some fish, *T. corlissi* were in the omental tissues of the peritoneal cavity, and in the meninges of the brain. Granulomas were in the liver with an increased number of melanomacrophage centres in several infected fish.

Histological examination of the three different types of skin lesion from fish submitted in January 2002 revealed differing pathology. The deep skin ulcers had similar pathology as described above, except that the ulcers extended only down to the level of the stratum compactum. Colonies of Gram negative bacteria covered the ulcerated surface and microcolonies were close to the edge of the ulcers. There were dilated blood vessels in the dermis, a generalised inflammatory infiltrate of mononuclear cells in the dermis and among the skeletal muscle and necrosis of the dermis and skeletal muscle fibres. *T. corlissi* were present in the loose connective tissues of the dermis. The paler areas of skin had epidermal hyperplasia with the epidermal layer 2 to 4 cells thicker than normal. The small dark marks visible on the caudal peduncle consisted of intact layer of epidermis. *T corlissi* were within epidermis and dermis and were associated with necrotic cells, infiltrates of mononuclear cells in the hypodermis and a few dilated blood vessels with haemorrhage. No colonies of bacteria were detected.

Fungi isolated from deep skin ulcers in May 2001 included *Curvularia* sp., *Fusarium* sp., *Paecilomyces* sp. and *Scopulariopsis* sp. These were considered secondary pathogens and will not be considered further. *Aeromonas sobria* was isolated from the skin ulcers, heart and caudal kidney of fish submitted in May 2001. *Aeromonas sobria* and *A. hydrophila* were isolated from the heart and from deep skin ulcers of fish submitted to OVL in July 2002.

7.3.4 Pond treatments and chemotherapeutic tests

Chemicals added to ponds during 2001 (Group B fish ponds) were ineffective in killing *T. corlissi.* $KMnO_4$ demand was 4 mg/L. All fish with skin lesions in ponds treated with $KMnO_4$ died by the following day and fish in ponds continued to die after both $KMnO_4$ and formalin treatments had been applied. *Tetrahymena* numbers on

lesions of dead and dying fish appeared to be unchanged, and the *Tetrahymena* were observed actively dividing and swimming throughout the treatment periods.

Bucket trials of fish infected with *T. corlissi*, were all ineffective in killing or reducing division activity of the parasite. Examination of wet mount preparations from skin scrapes done from the centre and margin of skin ulcers of affected fish showed presence of actively swimming and dividing *T. corlissi* during and after treatment.

Prolonged salt baths at 10‰ for 7 to 10 days were effective at killing exposed *T. corlissi*. After 3 days of salt immersion, the ulcers were not haemorrhagic and the skeletal muscle in the centre of the skin ulcers had turned white. Shallow skin scrapes done from ulcer margins showed no evidence of *T. corlissi,* however, deep scrapes taken from the ulcer margins showed small numbers of *T. corlissi.*

In the 2002 outbreak oral treatment with dimetridazole at 30 mg/kg of fish/day for 10 days showed cessation of mortalities within 3 days of treatment. Visual observations of fish medicated with Emytryl held in tanks showed healing of ulcers commenced within three days, and microscopic examination of scrapes found no *T. corlissi* in lesions.

7.4 Discussion

Both outbreaks of tetrahymenosis commenced during early winter when pond water temperatures dropped. In the outbreak of 2001 handling fish may have exacerbated an already stressed fish population and caused skin abrasion, predisposing fish to infection with *F. columnare*, *A. sobria* and *T. corlissi*. These fish growth trials were being done 900 km north of the natural distribution of golden perch. The fish were fed a diet for barramundi as no specific diet has been formulated for golden perch. Fish may have been immuno-compromised from low water temperatures or from a lack of particular essential nutrients. The natural distribution of golden perch is in areas with lower temperatures throughout winter, and the temperature tolerances of the fish are 4-37°C, suggesting that temperature is unlikely as the primary stressor. Fish may have had no effective immunity to local strains of *T. corlissi*, *Aeromonas* spp., and *F. columnare. Tetrahymena pyriformis* is well known for its genetic variability, and it is

possible that golden perch were naïve to the local *T. corlissi*. Jade perch (*Scortum barcoo*) kept in nearby ponds were also affected by tetrahymenosis (personal observation), whereas local species of fish also cultured on site were not. The appearance of infections in winter suggests that possibly *T. corlissi* is more active in colder weather, similar to *Ichthyophthirius multifiliis*.

In both the 2001 and 2002 outbreaks, *A. sobria* and *A. hydrophila* were identified as causing motile aeromonad septicaemia in the golden perch. Seasonal outbreaks of motile aeromonad septicaemia are often seen in stressed or immuno-compromised fish cultured in ponds (Roberts, 1993). *Aeromonas sobria* produces a potent enterotoxin (Carson, 1990), contributing to rapid necrosis of skin and muscle. Once motile aeromonads have invaded the integument of a compromised fish host, bacterial septicaemia can develop and result in rapid death. Lesions initiated by *T. corlissi* resulted in rapid sloughing of epidermis and destruction of dermal tissue. The rapidity of this was evidenced by the absence of, or poor immune response in the fish. Secondary bacterial infection then could have ensued, and the resultant toxins, haemorrhage, osmotic stress and septicaemia resulted in death of the fish.

The 2002 outbreak of tetrahymenosis occurred in a pond in which probiotics were used. Although *A. sobria* was not isolated from established skin lesions, *A. hydrophila* was isolated from deep skin lesions. The probiotics used in this case are used in crustacean aquaculture to reduce populations of *Vibrio* and viruses (Moriarty, 1998; Gatesoupe, 1999). The less acute nature of the 2002 outbreak may indicate that the probiotics were effective in reducing susceptibility to infection with *A. sobria* and *A. caviae*. In the previous six years, *A. sobria* has been isolated from all golden perch from ponds submitted for veterinary examination. This implies that the probiotic treatment impacted on pathogenic bacteria in the pond. It did not, however, impact on the *Tetrahymena* infection which was equally as devastating.

In this case, *T. corlissi* was differentiated from other *Tetrahymena* spp. previously described as opportunistic parasites of freshwater fish on the basis of morphological features. *T. pyriformis* is smaller in size (40 x 20 µm cf. 60 x 40 µm), possesses fewer meridional kineties (17-21 cf. 25-30) and does not possess a caudal cilium. *T.*

rostrata is larger and has more meridional kineties (32-48 cf. 25-30) including up to 4 postoral kineties (cf. 2).

The clinical signs of lethargy, gross and histological deep skin ulceration and internal invasion of organs and tissues are similar to those described for guppies, *Poecilia reticulata*, dwarf gouramis and other tropical aquarium fishes (Hoffman *et al.*, 1975; Imai *et al.*, 2000; Wakita *et al.*, 2002). The only epizootic involving *Tetrahymena* sp. in Australia affected silver perch grown in earthen ponds (Callinan and Rowland, 1994). The *Tetrahymena sp*. penetrated beneath the scales and invaded the skeletal muscle, causing scale lifting, skin ulceration, muscle swelling and necrosis. That study did not report the haemorrhagic margins of the lesions, which were a feature of the epizootic at FFAC.

The origin of the *T. corlissi* pathogenic to golden perch remains unknown. However several *Tetrahymena* sp. were detected from the gill filaments of dead tadpoles that cohabited the same pond as golden perch. *T. corlissi* does infect amphibians and there were several species of native frog present in the ponds at FFAC in which golden perch were reared. Golden perch may have been immunologically naïve to the local strain of *T. corlissi*. Several species of indigenous, native freshwater fishes (sleepy cod, *Oxyeleotris lineolatus*; barramundi, *Lates calcarifer*; long finned eels *Anguilla reinhardtii*) cultured in earthen ponds at FFAC, using the same water supply, were unaffected. Species of indigenous, native fish living in the settlement ponds where water drained from ponds containing golden perch affected *T corlissi*, including rainbow fish, *Melanotaenia splendida*, sleepy cod and hardyheads, *Craterocephalus stercusmuscarum* were also unaffected. One other non-indigenous but native fish species, jade perch, was cultured in an adjacent pond was also affected by *T. corlissi* and *A. sobria*. It is possible that native freshwater fish species had innate immunity to *T. corlissi, A. sobria* and *A. hydrophila.*

We are uncertain whether the *A. sobria* or *T. corlissi* was the primary pathogen responsible for the deep skin ulcers in the 2001 outbreak, since both pathogens were isolated from the ulcers and both are capable of producing deep skin ulcers with red margins in fish (Roberts, 1993; Thune *et al.*, 1993; Imai *et al.*, 2000). *Tetrahymena pyriformis* can carry viable bacteria into fish wounds in food vacuoles and discharge

them (King and Shotts, 1988). If *T. corlissi* did this it would increase the virulence spread and speed of death by facilitating spread of bacterial infection. However, in 2002 histological examination of fish with skin lesions consisting of paler than normal areas of intact skin, showed *T. corlissi* in the epidermis and dermis, underneath the intact epithelium, and no bacterial colonies were discernable. It is therefore apparent that *T. corlissi* can penetrate the intact skin, possibly through the scale pockets. *Tetrahymena corlissi* is histophagous and invades the scale pockets in other species of fish (Imai *et al.*, 2000).

In the 2001 outbreak fish were handled with nets in winter, and we believe this caused stress and skin abrasion resulting in loss of the protective mucous coat, allowing invasion by *T. corlissi, A. sobria* and *F. columnare*. However in the 2002 outbreak, fish were not handled in winter, yet similar skin lesions appeared. Only *A. hydrophila* was isolated from advanced skin ulcers in 2002, and not from the small red pinpoint skin lesions. *T. corlissi* was detected on areas of apparently healthy skin, and in the scale pockets and subcutaneous tissues, further suggesting that *T. corlissi* was the primary pathogen in the 2002 outbreak.

Callinan and Rowland (1995) implicated high organic loads in ponds as a precursor for tetrahymenosis in cultured silver perch. However, this was not apparent in all affected ponds at FFAC where some ponds had low fish densities and were comparatively clean, but still had high mortality.

The *T. corlissi* infecting golden perch appeared to be resistant to all bath treatments of chemicals tested. Callinan and Rowland (1995) recommended 10 g/L salt for 60 mins or 25 mg/L formalin for treatment of tetrahymenosis in silver perch reared in tanks. However we found salt and formalin baths were ineffective against *T. corlissi*. Our experience indicated that only long term bath treatments were effective against the surface dwelling *Tetrahymena*, but not the deep tissue infection. In our case, *T. corlissi* had invaded through the skin and infected internal organs and tissues, indicating treatment with a systemic drug. Indeed, the only treatment effective in treating *T. corlissi* infection was orally administered systemic anti-protozoal drug Emytryl (400 mg/g dimetridazole) at 30 mg/kg/fish/day. This drug was effective within 3 days of treatment as mortalities ceased in ponds, and healing of ulcers was

observed in fish in tanks. Dimetridazole acts by inhibiting DNA synthesis in aerobic bacteria (MIMS, 2003) and has been used effectively to control other motile internal protozoal infections such as *Hexamita* spp. (Hoffman and Meyer, 1974; Gratzek, 1993).

The challenge presented by *A. sobria* and *T. corlissi* is significant to golden perch culture. Both are ubiquitous, and as such present a potential threat to successful pond culture, particularly if fish are introduced and are naïve to a local strains of *Aeromonas* spp. or *Tetrahymena* spp. Our results showed that the *T. corlissi* present on golden perch was resistant to most commonly used ectoparasite chemotherapeutants. Results from the outbreak in 2002 showed that dimetridazole administered orally is effective against *T. corlissi*. In Australia dimetridazole is not registered for use on food fish, and its use in this trial was off label under direction of a veterinarian. None of the treated fish were used as food.

Chapter 8
General Conclusion

Weaning of golden perch is not as difficult as previously thought but does appear to require several conditions to be met. The length of the weaning period and the use of co-feeding diet attractive to golden perch appeared to be essential. Taste of the co-feeding diet is probably critical in assisting habituation otherwise the fingerlings refuse food altogether. Weaning using frozen zooplankton or *Artemia* as a co-feeding diet is easy and relatively cheap. *Artemia* can be purchased ready to use as frozen product from aquarium supply stores, thawed for mixing into blocks, and refrozen for use as required. Other sea foods require more processing to make them workable. The lack of disturbance while ice blocks melt might be one reason why this technique works well, although the fish do become habituated and associate the activity of putting the bags into the tanks, with food.

Density did have an effect on growth in the small experimental tanks. However, due to the short duration of the weaning period this may not be critical if the fish are to stocked into growout facilities immediately after weaning. Fingerlings for the nursery experiments in this study were weaned in 2 tonne tanks at densities of up to 4000 fish in 2000 L water, with success rates of over 90% survival. Up to 18,000 golden perch were weaned and any one time using this method, proving it could be employed on a large scale.

The effect of light on weaning demonstrated that low light intensity was ideal, producing better growth and weaning. Extending the results of that study to a growout system, combined with the results of the pond rearing studies, suggests that usual pond production system practices of promoting natural productivity may not be essential for culture of golden perch. This has important implications for development of aquaculture in inland Queensland where many earthen ponds or water storages are turbid with dispersive clays (primarily kaolin) which reduce primary production substantially. Maintaining oxygen levels in these situations requires diversion from

standard pond management practice, particularly if there is associated high biological oxygen demand with organic matter loads.

The ability of a small proportion of golden perch to wean successfully across to formulated food abruptly (about 13%) suggests that there is a proportion of fish which are opportunistic and eat whatever is available. Determination of whether these individuals are also the faster growing fish in a population would be a major contribution towards selective breeding for weaning ability and growth. Marking techniques using micro tagging are routinely used in wild fish and could probably be used for this fish. Additional follow up on whether early rapid growth of golden perch weaned at small sizes (19 mm, as opposed to 50 mm for stocking at present) is continued through to growout could also demonstrate whether this technique would be beneficial in a production situation. The catch up growth of point fed fish compared to broadcast fed fish in growout suggests that it would be beneficial, but further tests are required to confirm it. Production efficiencies in hatcheries could be increased substantially if fingerlings could be sold at a smaller size. Economies due to shorter production cycles make weaning at a smaller size an attractive option.

The testing of moist diets gave clear results, and suggests that golden perch feeding capacity is limited by physical capacity to hold food. This could indicate more regular feeding times at least in early culture. This is supported by the reduced growth in fish weaned using only two feeds per day instead of three. Trials on weaning of other fish species fed up to 40 times per day had increased growth rates compared to lower frequency feeding régimes. Feeding in ponds reduced from three times daily to afternoon feeds as fish grew, indicting that a regular or continuous feeding régime, if found practical, only need last a few months.

The results of these studies indicate that growth of golden perch free ranging in ponds is comparable in growth rate to other species of native Australian fish, if the bottom half of non performing fish are graded out. Previous studies on other perch and barramundi have studied growth after a graded sample of fish is stocked (Williams and Barlow, 1993; Rowland, 1995b). The indications of these experiments are that density has an effect on growth but only after about three months of juvenile growth. After that period growth of fish separates into those which grow rapidly and those that

do not. This growth appears to be partly mediated by sex. Feed selection also appears to be important, although not critical, in determining whether fish grow quickly. Those fish that retain feeding on pellets tend to grow faster than those which revert back to feeding on natural production in the ponds. The rate of reversion was reduced by exposing more fish to pellet food through broadcast feeding. However, some fish appeared to grow equally as well on natural food and, as observed in the tank trials on weaning, it is possible that a small proportion of fish are opportunistic and feed on whatever is available. As mentioned earlier, this allows a significant departure from traditional pond management in which natural productivity is promoted. It enables use of lower productivity water typical of water storages currently used solely for irrigation water storage in southern inland Queensland and northern New South Wales.

Growth potential appears to be retained and is apparently not suppressed by social factors, at least in the experimental conditions in this study. However, expression of growth potential seems to be restrained by other, unknown factors. It is not economical to retain fish which do not grow rapidly (i.e. to market size within 24 months). From the results of this study, about 50% of the faster growing fish should be retained for growout. The remainder could be sold on to other buyers who do not have economic imperatives to observe in growth rates of their fish, as these slower growing fish still have high growth potential which is not expressed in single species aquaculture ponds.

The high FCR is of concern as food is the highest operating cost in commercial aquaculture. The diet provided in these trials had a higher protein level than required by juvenile golden perch. A lower protein (40%) diet is about 10% lower in protein content than the diets for barramundi and salmon and thus could represent a significant reduction in feed costs. Investigation of nutritional requirements to produce a diet formulated specifically for golden perch should ameliorate this problem.

Feeding regimes may have a critical role to play in FCR. Feeding times matched to suit the fishes natural activity and feeding rhythms can have significant positive impacts on FCR and growth rates. Also, a fish such as golden perch which is adapted

to a feast or famine environment may differ from those species which inhabit stable environments. The apparent long residence time of food in starved golden perch could result in improved digestive efficiencies, so alteration of feeding regimes could have significant impacts on FCR. Feed efficiency can decrease rapidly as amount of food offered increases (Hung *et al.*, 1993), and experiments to assess food efficiency based on percentage body weight per day are necessary to determine the optimal rate of feeding which has no impact on growth. This has yet to be done for golden perch, so an optimal feeding rate that minimises wastage without compromising growth has yet to be determined. I suggest that feeding every second or third day could significantly improve FCR without compromising growth, thus improving economic viability of commercial production of golden perch for food.

The variability in growth rate between the different trials is not surprising considering the effect of parentage on growth rates and sex ratios. Variations in growth rates due to parentage vary from 3-15%, and can be due to differences in sex ratios or genetic factors. This variation again indicates a very high potential for selective breeding to obtain either favourable sex ratios and/or favourable growth rates (through a predilection for pellet foods).

Grading of golden perch did not appear to have a major effect of growth rates in this study. This result is in common with others that suggest that although grading is widespread in aquaculture it does not result in improved growth rates. Rather, it is a useful management tool to enhance efficiencies in feeding and harvesting. In golden perch, however, the disturbance caused during grading and the apparent selective mortality of faster growing females may outweigh the benefits in management. The apparent effect of disturbance on golden perch may be one reason for poor FCR, as after monthly sampling and water changes to control pH, food consumption dropped dramatically. Reducing disturbance to a minimum through less disruptive sampling techniques and not grading could improve production efficiencies in golden perch. The lack of a requirement for grading is certainly a positive factor in reducing labour inputs and handling. The observation that the smallest fish do not appear to eat pellets also makes them cheap to keep. As suggested earlier, smaller fish could be sold on for stocking purposes to recoup some costs.

Feeding of golden perch has been viewed as problematic by some farmers, as golden perch are bottom feeders, requiring a sinking pellet. However, prawns are bottom feeders and prawn aquaculture is one of the largest, most successful aquaculture industries in the world. Food trays are recommended for use in production of benthic animals such as prawns and redclaw to monitor feeding efficiency. Use of food trays for golden perch proved effective, although there were indications at times that feeding was selective on the trays, resulting in considerable overfeeding. Development of improved feeding techniques to reduce wastage could lead to substantial improvement in FCR and thus economic viability of farming golden perch.

The effect of sex ratios on growth suggests that sex manipulation would not be beneficial in golden perch production. The more rapid growth of females is only realised after the fish are close to or have reached market size. Initial market studies with smaller fish indicated a market for fish as small as 250 g (Graham, 2004a). All male culture would suit a spread of fish over the range of 250-700 g. However, females dominate the first fish reaching market size and would therefore contribute significantly to cash flow in a commercial situation.

Sexual maturation at under 12 months of age is not desirable in aquaculture. It raises questions as to whether maturity in the wild is driven by food availability or size of fish. We had female fish maturing after 8 months during the cooler part of the year, suggesting that temperature is not the prime factor influencing sexual maturity. Food availability may be more important than abiotic factors.

The problems encountered with the highly virulent *T. corlissi* infection demonstrate the challenges faced when growing a species of fish well outside its natural range in intensive culture for the first time. Only in the second year when it became apparent that the infection was primary and systemic was a systemic protozooicide trialed. This was found to be highly effective but in common with many other effective chemotherapeutics is not registered for use on food fish in Australia and so its use is strictly off label. The aetiology of the infection is still not completely understood- although *T. corlissi* is a primary pathogen the reasons why it attacks golden perch during the cool season are unclear. The fish should not be under temperature stress as cooler temperatures are experienced for much of the year in its native habitat.

Possibly the cooler temperatures favour the parasite and allow multiplication when naïve hosts are available. The absence of infection in indigenous fish stocks, and the finding of *Tetrahymena* in post mortem studies of golden perch and sleepy cod (only one or two per fish) further supports this. Identification and reduction or elimination of the stressor, which triggers *T. corlissi* attacking as a primary pathogen, could also lead to general production improvements.

Golden perch aquaculture appears to be viable given the high market prices which have been sustained over the years. The longer growing period and need for attention to some methodology would limit the number of farmers prepared to grow the species. However, it has many favourable attributes which mean that with appropriate management it should prove to be a viable, profitable option for freshwater fish growers to consider, particularly in areas with turbid water and limited natural productivity. The excellent flavour of golden perch regardless of the water they are taken from is an added bonus, removing the requirement for purging systems and resultant weight loss while purging. The highest priority for further research will be development of a species specific diet which makes use of the lower protein requirement and will improve FCR of this outstanding table fish.

Literature Cited

Abery, N.W., Gunasekaera, R.M. and de Silva, S.S. 2002. Growth and nutrient utilization of Murray Cod *Macchullochella peeli peeli* (Mitchell) fingerlings fed diets with varying levels of soybean meal and blood meal. *Aquaculture Research* **33**: 279-289.

Altinok, I. and Grizzle, J.M. 2001. Effects of low salinities on *Flavobacterium columnare* infection of euryhaline and freshwater stenohaline fish. *Journal of Fish Diseases* **24**: 361-367.

Alves, T.T., Cerquiera, V.R. and Brown, J.A. 2006. Early weaning of fat snook (*Centropomus parallelus* Poey 1864) larvae. *Aquaculture* **253**: 334-342.

Anderson, A.J. 1986. Nutrition and feeding of fish. In: Owen, P. and Bowden, J. (Eds.), *Freshwater Aquaculture in Australia*, pp. 25-31.

Anderson, D., Saoud, I.P. and Davis, D.A. 2002. The effects of stocking density on survival, growth, condition and feed efficiency of bluegill juveniles. *North American Journal of Aquaculture* **64**: 297-300.

Anderson, R.J. 1974. Feeding artificial diets to smallmouth bass. *Progressive Fish-Culturist* **36** (3): 145-151.

Anderson, T.A. and Braley, H. 1993. Appearance of nutrients in the blood of the golden perch *Macquaria ambigua* following feeding. *Comparative Biochemistry and Physiology* **104A** (2): 349-356.

Andrew, J.E., Holm, J. and Huntingford, F.A. 2004. The effect of pellet texture on the feeding behaviour of gilthead sea bream (*Sparus aurata* L.). *Aquaculture* **232** (1-4): 471-479.

Aneshansley, E.D. and Timmons, M.B. 2001. Survival and growth of walleye *Stizostedion vitreum* fry as affected by water recirculation, feed transition age, and stocking density. *Journal of the World Aquaculture Society* **32** (1): 89-95.

Anguas-Vélez, B.H., Civera-Cercedo, R., Contreras-Olguin, M., Rueda-Jauso, R.A. and Guillaume, J. 2000. Preliminary study on timing of weaning of spotted sand bass, *Paralabrax maculofasciatus*, larvae with a prepared diet: effects of growth and survival. *Journal of Applied Aquaculture* **10** (4): 1-16.

Anonymous 2003. Tracking golden perch in Lake Eildon. *Freshwater Fishing Australia* **63**: 15.

Arumugam, P.T. 1986a. An experimental approach to golden perch (*Macquaria ambigua*) fry-zooplankton interactions in fry rearing ponds, South-eastern Australia. Doctor of Philosophy Thesis. University of Adelaide: Adelaide.

Arumugam, P.T. 1986b. Importance of initial laboratory feeding for golden perch fry. The First Asian Fisheries Forum. Asian Fisheries Society, Manilla, Philippines pp. 529-531.

Arumugam, P.T. and Geddes, M.C. 1987. Feeding and growth of golden perch larvae and fry (*Macquaria ambigua* Richardson). *Proceedings of the Royal Society of South Australia* **111** (1-2): 59-65.

Arumugam, P.T. and Geddes, M.C. 1988. Predation of golden perch (*Macquaria ambigua*) fry on *Daphnia carinata* in nursery ponds, Australia. Proceedings of the International Association for Theoretical and Applied Limnology, Congress in New Zealand, New Zealand pp. 1773-1776.

Baardvik, B.M. and Jobling, M. 1990. Effect of size-sorting on biomass gain and individual growth rates in Arctic charr, *Salvelinus alpinus* L. *Aquaculture* **90** (1): 11-16.

Babiak, I., Mandiki, S.N.M., Ratsinjomanana, K. and Kestemont, P. 2004. Initial weight and its variation in post-larval Eurasian perch affect quantitative characteristics of juvenile cohorts under controlled conditions. *Aquaculture* **234** (1-4): 263-276.

Bancroft, J.D. and Stevens, A. 1990. *Theory and practice of histological techniques.* 3rd Edition. Churchill Livingstone, London.

Baras, E., Tissier, F., Westerloppe, L., Mélard, C. and Philippart, J. 1998. Feeding in darkness alleviates density-dependent growth of juvenile vundu catfish *Heterobranchus longifilis* (Clariidae). *Aquatic Living Resources* **11** (5): 335-340.

Baras, E. and Jobling, M. 2002. Dynamics of intracohort cannibalism in cultured fish. *Aquaculture Research* **33**: 461-479.

Barki, A., Harpaz, S., Hulata, G. and Karplus, I. 2000. Effects of larger fish and size grading on growth and size variation in fingerling silver perch. *Aquaculture International* **8** (5): 391-401.

Barki, A. and Karplus, I. 2004. Size rank and growth potential in redclaw crayfish (*Cherax quadricarinatus*): are stunted juveniles suitable for grow-out? *Aquaculture Research* **35**: 559-567.

Barlow, C.G., McLoughlin, R. and Bock, K. 1987. Complementary feeding habits of golden perch *Macquaria ambigua* (Richardson)(Percichthyidae) and silver perch *Bidyanus bidyanus* (Mitchell)(Teraponidae) in farm dams. *Proceedings of the Linnaean Society of New South Wales* **109** (3): 143-152.

Barlow, C.G., Rodgers, L.J., Palmer, P.J. and Longhurst, C.J. 1993. Feeding habits of hatchery reared barramundi *Lates calcarifer* (Bloch) fry. *Aquaculture* **109**: 131-144.

Barlow, C.G. and Rodgers, L.J. 1993. Optimal size for weaning barramundi *Lates calcarifer* fry onto artificial diets. Nutrition and Culture of Larval and Juvenile Barramundi. FRDC Project Final Report. FRDC Project. Queensland Department of Primary Industries: Brisbane, Australia. 6.1-6.8 pp.

Barnabe, G. 1991. *Acuicultura.* Omega, Barcelona, Spain.

Baroiller, J.-F. 1999. Endocrine and environmental aspects of sex differentiation in fish. *Cellular and Molecular Life Sciences* **55** (6-7): 910 - 931.

Baskerville-Bridges, B. and Kling, L.J. 2000. Early weaning of Atlantic cod (*Gadus morhua*) larvae onto a microparticulate diet. *Aquaculture* **189** (1-2): 109-117.

Battaglene, S. and Prokop, F. 1987. Golden Perch. Agfact F3.2.2. Department of Agriculture, New South Wales: Grafton. 7 pp.

Battaglene, S.C. 1991. The golden perch *Macquaria ambigua* (Pisces: Percichthyidae) of Lake Keepit, NSW. Master of Science Thesis. University of New South Wales: Sydney.

Beumer, J.P., Ashburner, L.D., Burbury, M.E., Jetté, E. and Latham, D.J. 1982. A checklist of the parasites of fishes from Australia and its adjacent Antarctic territories. *Commonwealth Agricultural Bureaux, Institute of Parasitology, Technical Communication*, **48**: 1-99.

Boeuf, G. and le Bail, P.-Y. 1999. Does light have an influence on fish growth? *Aquaculture* **177**: 129-152.

Bombeo-Tuburan, I., Coniza, E.B. and Rodriguez, E. 2002. Preliminary report on nursery and grow-out culture of hatchery-bred grouper (*Epinephelus coiodes* Hamilton) in ponds. *Aquaculture Research* **33**: 379-381.

Bondari, K. 1983. Training and growth of artificially fed largemouth bass in culture tanks. *Fisheries Management* **14** (3): 145-149.

Bowerman, M.R. 1975. Important discoveries about native warm-water fish. *Australian Fisheries* **34** (5): 3-8.

Brännäs, E. and Linnér, J. 2000. Growth effects in arctic charr reared in cold water: Feed frequency, access to bottom feeding and stocking density. *Aquaculture International* **8**: 381-389.

Bromley, P.J. and Smart, G. 1981. The effects of the major food categories on growth, composition and food conversion in rainbow trout (*Salmo gairdneri* Richardson). *Aquaculture* **23** (1-4): 325-336.

Brumley, A.R. 1987. Past and present distributions of golden perch *Macquaria ambigua* (Pisces: Percichthyidae) in Victoria, with references to releases of hatchery produced fry. *Proceedings of the Royal Society of Victoria* **99** (3): 111-116.

Busacker, G.P., Adelman, I.R. and Goolish, E.M. 1990. Growth. In: Schreck, C.B. and Moyle, P.B. (Eds.), *Methods for Fish Biology.* American Fisheries Society, Bethesda, pp. 363-387.

Cadwallader, P.L. and Backhouse, G.N. 1983. *A Guide to the Freshwater Fish of Victoria.* Victorian Government Printing Office, Melbourne, 249 pp.

Cadwallader, P.L. 1985. Freshwater fisheries production in Australia. *Australian Fisheries* **44** (9): 12-15.

Callinan, R.B. and Rowland, S.J. 1994. Diseases of Silver perch. Proceedings of Silver Perch Aquaculture Workshops, Grafton and Narrandera, Austasia Aquaculture pp. 67-75.

Cañavate, J.P. and Fernandez-Diaz, C. 1999. Influence of co-feeding larvae with live and inert diets on weaning the sole *Solea senegalensis* onto commercial dry feeds. *Aquaculture* **174**: 255-263.

Carillo, M., Zanuy, S., Prat, F., Cerda, J., Ramos, J., Mananos, E. and Bromage, N. 1995. Sea bass (*Dicentrarchus labrax*). In: Bromage, N. and Roberts, J. (Eds.), *Broodstock management and egg and larval quality.* Blackwell, Oxford, pp. 138-169.

Carragher, J.F. and Rees, C.M. 1994. Primary and secondary stress responses in golden perch, *Macquaria ambigua*. *Comparative Biochemistry and Physiology* **107A**: 49-56.

Carson, J. 1990. Bacterial infections of finfish. Fin Fish Diseases. Refresher Course for Veterinarians., University of Sydney, Post Graduate Foundation in Veterinary Science, University of Sydney pp. 56-72.

Carson, J., Schmidtke, L.M. and Munday, B.L. 1993. *Cytophaga johnsonae*: a putative skin pathogen of juvenile farmed barramundi, *Lates calcarifer* Bloch. *Journal of Fish Diseases* **16**: 209-218.

Chandrika, V. 1996. Incidence of antagonistic bacillus spp.- An eco-friendly aquatic probiotic from aquaculture ponds. The Fourth Indian Fisheries Forum, 24-28 Nov 1996., Kochi, Jerala pp. 147-150.

Chesney, E.J. 1989. Estimating food requirements of striped bass larvae *Morone saxatilis*: effects of light, turbidity and turbulence. *Marine Ecology Progress Series* **53**: 191-200.

Chowdhury, M.B.R. and Wakabayashi, H. 1991. A study on *Flexibacter columnaris* infection in loach, *Misgurnus anguillicaudatus* (Bleeker, Guenther). *Journal of Fish Diseases* **14** (3): 389-394.

Chude, L.A. 2001. The effects of crowding stress, different diets and different size classes on the growth of *Clarias gariepinus*. *Journal of Aquatic Sciences* **16**: 70-75.

Collins, A. 1996. Physiological changes associated with feeding frequency in the Golden Perch, *Macquaria ambigua* (Richardson)(Pisces: Percichthyidae). Ph. D. James Cook University of North Queensland: Townsville.

Collins, A. and Anderson, T. 1999. The role of food availability in regulating reproductive development in female golden perch. *Journal of Fish Biology* **55** (1): 94-104.

Collins, A.L. and Anderson, T.A. 1995. The regulation of endogenous energy stores during starvation and refeeding in the somatic tissues of the golden perch. *Journal of Fish Biology* **47**: 1004-1015.

Coutteau, P. and Sorgeloos, P. 1997. Manipulation of dietary lipids, fatty acids and vitamins in zooplankton cultures. *Freshwater Biology* **38** (3): 501-512.

Crook, D.I., Robertson, A.I., King, A.I. and Humphries, P. 2001. The influence of spatial scale and habitat arrangements of diel patterns of habitat use by two lowland river fishes. *Oecologia* **129** (4): 525-533.

Cuzon, G., Coatanea, D., Mélard, J., Peignon, J.M., Bard, F.X., Bessineton, C., Calvas, J., Griessinger, J.M., Fallourd, F., Lorin, G., Mailion, A., Le Bitoux, J.F., Martin, J.L., Fontanabona, A. and Michel, A. 1975. First experiments in rearing of tropical Carangidae in floating cages. Sixth Annual Meeting of the World Mariculture Society 1975, Seattle, USA, World Mariculture Society pp. 277-284.

Dabrowski, K., Charlon, N., Berget, P. and Kaushik, S.J. 1984. Rearing of coregonid (*Coregonus schenzi palea* Cuv. et Val.) larva using dry and live food. 1. Preliminary data. *Aquaculture* **41**: 11-20.

Dabrowski, K.R. and Rusiecki, M. 1983. Content of total and free amino acids in zooplanktonic food of fish larvae. *Aquaculture* **30** (1-4): 31-42.

De Silva, S., Gunasekera, R.M., Ingram, B.A. and Dobson, J. 2001. Weaning of Australian shortfin eels (*Anguilla australis*): a comparison on the effectiveness of four types of fish roe. *Aquaculture* **195**: 133-148.

De Silva, S., Gunasekera, R., Collins, R. and Ingram, B.A. 2002. Performance of juvenile Murray cod, *Maccullochella peelii peelii* (Mitchell), fed with diets of different protein to energy ratio. *Aquaculture Nutrition* **8** (2): 79-85.

De Silva, S.S., Gunasekera, R. and Collins, B. 2000. Synopsis on nutrition research on Murray cod. Murray Cod Aquaculture, a Potential Industry for the New Millenium,

Eildon (Australia), 18 Jan 2000, Eildon, Victoria, VDNRE, Melbourne, Vic. pp. 30-33.

Denson, M.R. and Smith, T.I.J. 1996. Larval rearing and weaning techniques for white bass *Morone chrysops*. *Journal of the World Aquaculture Society* **27** (2): 194-201.

Devaraja, T.N., Yusoff, F.M. and Shariff, M. 2002. Changes in bacterial populations and shrimp production in ponds treated with commercial microbial products. *Aquaculture* **206**: 245-256.

Devlin, R.H. and Nagahama, Y. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. *Aquaculture* **208** (3-4): 191-364.

Devresse, B., Candreva, P., Leger, P., Sorgeloos, P., Lavens, P., Sorgeloos, P., Jaspers, E. and Ollevier, F. 1991. A new artificial diet for the early weaning of seabass (*Dicentrarchus labrax*) larvae. Fish and Crustacean Larviculture Symp., Gent (Belgium),, Gent (Belgium), European Aquaculture Society pp. 178-182.

Diver, L. 2000. Fish stocking frenzy. *Fisheries N.S.W.* **3** (1): 8-9.

Edgerton, B., O'Donoghue, P., Wingfield, M. and Owens, L. 1996. Systemic infection of freshwater crayfish *Cherax quadricarinatus* by hymenostome ciliates of the *Tetrahymena pyriformis* complex. *Diseases of Aquatic Organisms* **27**: 123-129.

Efthimiou, S., Divanach, P. and Rosenthal, H. 1994. Growth, food conversion and agonistic behaviour in common dentex (*Dentex dentex*) juveniles fed on pelleted moist and dry diets. *Aquatic living resources* **7** (4): 267-275.

Efthimiou, S. 1996. Performance of juvenile and ongrowing common dentex (*Dentex dentex*, L. 1758, Sparidae) in relation to nutrition under culture. *Berichte aus dem Institut fur Meereskunde an der Christian-Albrechts-Universitat Kiel.* **286**: 123.

Endemann, F., Kaiser, H. and Hecht, T. 1997. Size sorted juvenile swordtails, *Xiphophorus helleri* Heckel 1848 (Family Poeciliidae), do not show improved growth. *Aquaculture Research* **28** (8): 645-647.

Esteban, M.A., Mulero, V., Ortuno, J., Cuesta, A. and Meseguer, J. 1999. Vitamins as immunostimulants of farmed seabream. 7. National Congress of Aquaculture. 7. Congreso Nacional de Acuicultura, Las Palmas (Gran Canaria), 19-21 May 1999, Instituto Canario de Ciencias Marinas ICCM, Direccion General de Universidades E Investigacion Consejeria de Edicacion Cultura y Deportes Gobierno de Canarias.

Fallu, R. and Mosig, J. 1994. *Australian Fish Farmer. A Practical Guide to Aquaculture.* Agmedia, Melbourne.

Ferguson, H.W., Hicks, B.D., Lynn, D.H., Ostland, V.E. and Bailey, J. 1987. Cranial ulceration in Atlantic salmon *Salmo salar* associated with *Tetrahymena* sp. *Diseases of Aquatic Organisms* **2**: 191-195.

Flickinger, S.A., Anderson, R.J. and Puttmann, S.J. 1975. Intensive culture of smallmouth bass. Black bass biology and management, National Symposium on the Biology and Management of the Centrarchid Basses, Oklahoma.

Flores, H., Oritz, G. and Silva, A. 1994. Water stability of moist feeds for Chilean flounder. *Aquaculture* **124** (1-4): 287.

Frances, J., Nowak, B.F. and Allan, G.L. 2000. Effects of ammonia on juvenile silver perch (*Bidyanus bidyanus*). *Aquaculture* **183**: 95-103.

Francis, R.C. and Barlow, G.W. 1993. Social control of primary sex differentiation in the Midas cichlid. *Proceedings of the National Academy of Science U.S.A.* **90**: 10673-10675.

Gatesoupe, F.J. 1999. The use of probiotics in aquaculture. *Aquaculture* **180** (1-2): 147-165.

Gennari, L., Roncarti, A., Melotti, P. and Loro, F. 1994. Early weaning trials of sea bass (*Dicentrarchus labrax* L.) using different artificial diets. *Aquaculture* **124**: 283-284.

Ginetz, R.M. and Larkin, P.A. 1973. Choice of colors of food items by rainbow trout (*Salmo gairdneri*). *Journal of the Fisheries Research Board, Canada* **30**: 229-234.

Glencross, B.D., van Barneveld, R.J., Carter, C.G. and Clarke, S.M. 1999. On the path to a manufactured feed for farmed southern bluefin tuna. *World Aquaculture* **30** (3): 42-47.

Glencross, B.D., Clarke, S.M., Buchanan, J.G., Carter, C.G. and van Barneveld, R.J. 2002. Temporal Growth Patterns of Farmed Juvenile Southern Bluefin Tuna, Thunnus maccoyii (Castelnau) Fed Moist Pellets. *Journal of the World Aquaculture Society* **33** (2): 138-145.

Gomez-Gil, B., Roque, A. and Turnbull, J.F. 2000. The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. *Aquaculture* **191**: 259-270.

Gornati, R., Terova, G., Vigetti, D., Prati, M., Saroglia, M. and Bernardini, G. 2004. Effects of population density of seabass (*Dicentrarchus labrax*, L.) gene expression. *Aquaculture* **230**: 229-239.

Goudie, C.A., Simco, B.A., Davis, K.B. and Carmichael, G.J. 1993. Size grading may alter sex ratios of fingerling channel catfish. *Progressive Fish-Culturist* **55** (1): 9-15.

Graham, P. 2004a. Marketing of farmed golden perch. *Austasia Aquaculture* **18** (3): 48-49.

Graham, P. 2004b. Harvesting zooplankton as a food source for larvae and fingerlings. *Austasia Aquaculture* **18** (3): 56-57.

Gratzek, J.B. 1993. Parasites associated with freshwater tropical fishes. In: Stoskopf, M.K. (Ed.), *Fish Medicine*. WB Saunders Company, Sydney., pp. 573-590.

Grove, D., Genna, R., Paralika, V., Boraston, J., HornyoldM, G. and Siemens, R. 2001. Effects of dietary water content on meal size, daily food intake, digestion and growth in turbot, *Scophthalmus maximus* (L.). *Aquaculture Research* **32** (6): 433-442.

Gunasekera, R.M., Gooley, G.J. and De Silva, S.S. 1998. Characterisation of `swollen yolk-sac syndrome' in the Australian freshwater fish Murray cod, *Maccullochella peelii peelii*, and associated nutritional implications for large scale aquaculture. *Aquaculture* **169** (1-2): 69-85.

Gunasekera, R.M., De Silva, S.S., Collins, R.A., Gooley, G. and Ingram, B.A. 2000. Effect of dietary protein level on growth and food utilization in juvenile Murray cod *Maccullochella peelii peelii* (Mitchell). *Aquaculture Research* **31** (2): 181-187.

Hamlyn, A. and Cheetham, R. 2001. Post stocking survey report: Callide Dam, Biloela, survey 3, 20 April 2001 . Information Series QI01059. Queensland Fisheries Service, Department of Primary Industries Brisbane, Qld. Australia: 12 pp.

Harpaz, S., Hulata, G., Karplus, I. and Segev, R. 2001. Effect of density on the growth and survival of juvenile silver perch (*Bidyanus bidyanus*, Mitchell), under intensive production conditions. 6th Asian Fisheries Forum Book of Abstracts., Kaohsiung (Taiwan), Asian Fisheries Society pp. 92.

Hatanaka, H. 1997. Influence of tank colour, light intensity and rearing density on the growth rate and the shape of caudal fin in juvenile tiger puffer *Takifugu rubripus*. *Nippon Suisan Gakkaishi* **63**: 734-738.

Henderson-Arzapalo, A. and Stickney, R.R. 1982. Effects of stocking density on two tilapia species raised in an intensive culture system. Proceedings of the Annual Conference of Southeast Association of Fish and Wildlife Agencies.

Herbert, B.W. and Graham, P.A. 2004. Use of *Artemia*, Frozen Zooplankton and Artificial Food for Weaning Fingerlings of the Freshwater Fish Golden Perch *Macquaria ambigua ambigua* (Percichthyidae). *Asian Fisheries Science* **16** (1).85-90

Higuera, M. 2001. Chapter 11. Effects of nutritional factors and feed characteristics on feed intake. In: Houlihan, D., Boujard, T. and Jobling, M. (Eds.), *Food intake in Fish.* Blackwell Science Ltd, Oxford, pp. 418.

Hilge, V. and Steffens, W. 1996. Aquaculture of fry and fingerlings of pike - perch (*Stizostedion lucioperca* L.) - a short review. *Journal of Applied Ichthyology* **12** (3 - 4): 167-170.

Hoffman, G.L. and Meyer, F.P. 1974. *Parasites of freshwater fishes. A review of their control and treatment.* TFH Publications, Neptune City, New Jersey.

Hoffman, G.L., Landolt, M., Camper, J.E., Coats, D.W., Stookey, J.L. and Burek, J.D. 1975. A disease of freshwater fishes caused by *Tetrahymena corlissi* Thompson, 1955, and a key for identification of holotrich ciliates of freshwater fishes. *Journal of Parasitology* **61** (2): 217-223.

Humphries, P., King, A.J. and Koehn, J.D. 1999. Fish, flows and floodplains: links between freshwater fishes and their environment in the Murray-Darling River system, Australia. *Environmental Biology of Fishes* **56**: 129-151.

Hung, S.S.O., Conte, F.S. and Hallen, E.F. 1993. Effects of feeding rates on growth, body composition and nutrient metabolism in striped bass (*Morone saxatilis*) fingerlings. *Aquaculture* **112**: 349-361.

Huse, I., Bjordal, A., Fernö, A. and Furevik, D. 1990. The effect of shading in pen-reared Atlantic salmon (*Salmo salar*). *Aquaculture Engineering* **9**: 235-244.

Iguchi, K., Ogawa, K., Nagae, M. and Ito, F. 2003. The influence of rearing density on stress response and disease susceptibility of ayu (*Plecoglossus altivelis*). *Aquaculture* **220**: 515-523.

Imai, S., Tsurimaki, S., Goto, E., Wakita, K. and Hatai, K. 2000. *Tetrahymena* infection in guppy, *Poecilia reticulata*. *Fish Pathology* **35**: 67-72.

Ingram, B.A. 2000. Murray Cod aquaculture: A potential industry for the new millennium. Proceedings of a workshop held 18th January 2000., Eildon, Victoria, Department of Natural Resources and Environment, Marine and freshwater Resources Institute, Victoria. pp. 42.

Jenkins, W.E. and Smith, T.I.J. 1999. Pond nursery production of southern flounder (*Paralichthys lethostigma*) and weaning to commercial diets. *Aquaculture* **176**: 173-180.

Jobling, M. 1983. Growth studies in fish-overcoming the problems of size variation. *Journal of Fish Biology* **22**: 153-157.

Jodun, W.A., Millard, M.J. and Mohler, J. 2002. The effect of rearing density on growth, survival and feed conversion of juvenile Atlantic sturgeon. *North American Journal of Aquaculture* **64**: 10-15.

Jones, D.A., Kamarudin, M.S., Le Vay, L., Lavens, P., Sorgeloos, P., Jaspers, E. and Ollevier, F. 1991. The potential for replacement of live feeds in larval culture. Fish and Crustacean Larviculture Symp., Gent (Belgium), Gent (Belgium), European Aquaculture Society pp. 141-143.

Jory, D.E. 1998. Use of probiotics in Penaeid shrimp growout. *Aquaculture Magazine* **24**: 62-67.

Juario, J.V., Duray, M.N. and Fuchs, J. 1991. Weaning of seabass, *Lates calcarifer* B., larvae to artificial diet. Larvi '91 - Fish and Crustacean Larviculture Symposium., Ghent, Belgium, European Aquaculture Society pp. 183.

Juell, J.-E. and Lekang, O.I. 2001. The effect of feed supply rate on growth of juvenile perch (*Perca fluviatilis*). *Aquaculture Research* **32**: 459-464.

Kaiser, H., Weyl, O. and Hecht, T. 1995. Observations on agonistic behaviour of *Clarius gariepinus* larvae and juveniles under different densitites and feeding frequencies in a controlled environment. *Journal of Applied Ichthyology* **11**: 25-36.

Keenan, C., Watts, W. and Serafini, L. 1995. Population genetics of golden perch,. silver perch and eel tailed catfish within the Murray-Darling basin. Proceedings of the Murray Darling Basin Commission Riverine Environment Research Forum, Attwood Victoria, Murray Darling Basin Commission. Natural Resources Management Strategy pp. 17-26.

Kestemont, P., Mélard, C., Fiogbe, E., Vlavonou, R. and Masson, G. 1996. Nutritional and animal husbandry aspects of rearing early life stages of Eurasian perch *Perca fluviatilis*. *Journal of Applied Ichthyology* **12** (304): 157-165.

Kestemont, P., Jourdan, S., Houbart, M., Mélard, C., Paspatis, M., Fontaine, P., Cuvier, A., Kentouri, M. and Baras, E. 2003. Size heterogeneity, cannibalism and competition in cultured predatory fish larvae: biotic and abiotic influences. *Aquaculture* **227** (1-4): 333-356.

King, C.H. and Shotts, E.B. 1988. Enhancement of *Edwardsiella tarda* and *Aeromonas salmonicida* through ingestion by the ciliated protozoan *Tetrahymena pyriformis*. *Federation of European microbiological Societies Microbiology Letters* **51**: 95-100.

Kohda, M. 1994. Individual specialized foraging repertoires in the piscivorous cichlid fish *Lepidolamprologus profundicola*. *Animal Behaviour* **48**: 1123-1131.

Kolkovski, S. 2001. Digestive enzymes in fish larvae and juveniles--implications and applications to formulated diets. *Aquaculture* **200** (1-2): 181-201.

Koskela, J., Pirhonen, J. and Virtanen, E. 1991. Effect of feed attractants on feed choice of rainbow trout, *Oncorhynchus mykiss*. In: Kanshik, S.J. and Luquest, P. (Eds.), *Fish Nutrition in Practice.* Institut National de la Recherche Agronomique, Paris, pp. 419-427.

Kubitza, F. and Lovshin, L.L. 1997. The use of freeze-dried krill to feed train largemouth bass (*Micropterus salmoides*): feeds and training strategies. *Aquaculture* **148**: 299-312.

Kubitza, F. and Lovshin, L.L. 1999. Formulated diets, feeding strategies, and cannibalism control during intensive culture of juvenile carnivorous fishes. *Reviews in Fisheries Science* **7** (1): 1-22.

Lake, J.S. 1967. Freshwater Fish of the Murray-Darling River System. *State Fisheries of New South Wales, Research Bulletin* **7**: 48.

Lake, J.S. 1971. *Freshwater Fishes and Rivers of Australia.* Thomas Nelson, Melbourne, 61 pp.

Lambert, Y. and Dutil, J.-D. 2001. Food intake and growth of adult Atlantic cod (*Gadus morhua* L.) reared under different conditions of stocking density, feeding frequency and size-grading. *Aquaculture* **192** (2-4): 233-247.

Lee, E.M. 1988. Commercial cod farming operations, Newfoundland, 1988. (Canada). *Canadian Industry Report of Fisheries and Aquatic Sciences* **201**: 1-59.

Lee, G.Y.W. and Litvak, M.K. 1996. Weaning of wild young of the year winter flounder *Pleuronectes americanus* (Walbaum) on a dry diet: Effects on growth, survival, and feed efficiency ratios. *Journal of the World Aquaculture Society* **27** (1): 30-39.

Liang, X.F., Oku, H., Ogata, H.Y. and He, X. 2001. Weaning Chinese perch *Siniperca chuatsi* (Basilewsky) onto artificial diets based on its specific sensory modality in feeding. *Aquaculture Research* **32** (1): 76-82.

Lin, C.K. and Yi, Y. 2003. Minimizing environmental impacts of freshwater aquaculture and reuse of pond effluents and mud. *Aquaculture* **226**: 57-68.

Ljunggren, L., Staffan, F., Falk, S., Linden, B. and Mendes, J. 2003. Weaning of juvenile pikeperch, *Stizostedion lucioperca* L., and perch, *Perca fluviatilis* L., to formulated feed. *Aquaculture Research* **34** (4): 281-287.

Llewellyn, C. and MacDonald, C.M. 1980. Family Percichthyidae. Australian Freshwater basses and cods. In: McDowall, R.M. (Ed.), *Freshwater Fishes of South-eastern Australia.* A.H. and A.W. Reed Pty Ltd., Sydney, pp. 142-149.

Logan, D.E. 1986. Investigation of the lysine requirement of golden perch (*Macquaria ambigua*). B. App. Sc. Queensland Institute of Technology: Brisbane.

Lom, J. 1984. Diseases caused by protistans. In: Kinne, O. (Ed.), *Diseases of Marine Animals Volume IV, Part 1. Introduction Pisces.* Biologische Anstalt Helgoland, Hamburg, Germany, pp. 114-168.

Lom, J. and Dyková, I. 1992. Ciliates (Phylum Ciliophora Dofelin, 1901). In: Lom, J. and Dyková, I. (Eds.), *Protozoan Parasites of Fishes*. Elsevier, Amsterdam, pp. 237-288.

Lovric, D. 2000. Weaning and rearing of juvenile Murray Cod. Murray Cod Aquaculture: A Potential Industry for the New Millennium. Proceedings of a Workshop held 18th January 2000, Eildon, Victoria, Eildon, Victoria, Marine and Freshwater Resources Institute, Victoria. pp. 33.

Malison, J.A., Kayes, T.B., Wenthworth, B.C. and Amundson, C.H. 1988. Growth and feeding response of male versus female yellow perch (*Perca flavescens*) treated with estradiol-17 beta . *Canadian Journal of Fisheries and Aquatic Sciences* **45**: 1942-1948.

Malison, J.A. and Held, J. 1992. Effects of fish size at harvest, initial stocking density and tank lighting conditions on the habituation of pond-reared yellow perch (*Perca flavescens*) to intensive culture conditions. *Aquaculture* **104**: 67-78.

Maneewong, S. 1987. Research on the nursery stages of sea bass (*Lates calcarifer*) in Thailand. Management of wild and cultured sea bass/barramundi (*Lates calcarifer*): Proceedings of an international workshop held at Darwin, N.T. Australia 24-30 September 1986., Darwin, ACIAR Proceedings pp. 138-141.

Maragoudaki, D., Paspatis, M. and Kentouri, M. 1999. Influence of stocking density on growth of juvenile red porgy *Pagrus pagrus* L. under different feeding conditions. *Aquaculture Research* **30**: 501-508.

Marsden, G.E., McGuren, J.J., Hansford, S.W. and Burke, M.J. 1997. A moist artificial diet for prawn broodstock: its effect on the variable reproductive performance of wild caught *Penaeus monodon*. *Aquaculture* **149** (1-2): 145-156.

Marshall, T.C., Grant, E.M. and Haysom, N.M. 1959. *Know Your Fishes.* Department of Harbours and Marine, Brisbane.

Martins, C.I.M., Aanyu, M.M., Schrama, J.W. and Verreth, J.A.J. 2005. Size distribution in African catfish (*Clarias gariepinus*) affects feeding behaviour but not growth. *Aquaculture* **250** (1-2): 300-307.

Martins, C.I.M., Schrama, J.W. and Verreth, J.A.J. 2006. The effect of group composition on the welfare of African catfish (Clarias gariepinus). *Applied Animal Behaviour Science* **97** (2-4): 323-334.

Martins, C.I.M., Schrama, J.W. and Verreth, J.A.J. in press. Inherent variation in growth efficiency of African catfish Clarias gariepinus (Burchell 1822) juveniles. *Aquaculture Research*.

Masterson, M.F. and Garling, D.L. 1986. Effect of feed colour on feed acceptance and growth of walleye fingerlings. *Progressive Fish-Culturist* **48**: 306-309.

McFayden, P. 2001. An Investigation into the Protein Requirements for Growth in Golden Perch. Bachelor of Agricultural Science, Final Year Project. University of Queensland: Gatton.

Mélard, C., Baras, E., Mary, L. and Kestemont, P. 1996a. Relationships between stocking density, growth, cannibalism and survival rate in intensively cultured larvae and juveniles of perch (*Perca fluviatilis*). *Annales Zoologici Fennici* **33**: 643-651.

Mélard, C., Kestemont, P. and Grignard, J.C. 1996b. Intensive culture of juvenile and adult Eurasian perch (*P. fluviatilis*): effect of major biotic and abiotic factors on growth. *Journal of Applied Ichthyology* **12**: 175-180.

Merrick, J.R. 1976. The waters and fish of the Lake Eyre basin. Part II. *The Royal Society of New South Wales, Newsletter.* (14).

Merrick, J.R. and Schmida, G.E. 1984. *Australian Freshwater Fishes.* John R. Merrick, Netley, 409 pp.

Moriarty, D.J.W. 1998. Control of luminous *Vibrio* species in penaeid aquaculture ponds. *Aquaculture* **164**: 351-358.

Mosig, J. 2001. Pond culture and recirculation a profitable combination for golden perch grower. *Austasia Aquaculture* **15** (1): 32-35.

Mosig, J. 2002. Research on sleepy cod in North Queensland, Australia. *Hatchery International* **3** (2): 4-8.

Moura, M.A., Kubitza, F. and Cyrino, J.E. 2000. Feed training of peacock bass (*Cichla* sp.). *Revista Brasiliera de Biologia* **60** (4): 645-654.

Mourente, G., Diaz-Salvago, E., Bell, J.G. and Tocher, D.R. 2002. Increased activities of hepatic antioxidant defence enzymes in juvenile gilthead sea bream (*Sparus aurata* L.) fed dietary oxidised oil: attenuation by dietary vitamin E. *Aquaculture* **214** (1-4): 343-361.

Musyl, M.K. and Keenan, C.P. 1992. Population genetics and zoogeography of Australian freshwater golden perch, *Macquaria ambigua* (Richardson 1845)

(Teleostei: Percichthyidae), and electrophoretic identification of a new species from the Lake Eyre basin. *Australian Journal of Marine and Freshwater Research* **43**: 1585-1601.

Næss, T., Hamre, K. and Holm, J.C. 2001. Successful early weaning of Atlantic halibut (*Hippoglossus hippoglossus* L.) in small shallow raceway systems. *Aquaculture Research* **32** (3): 163-168.

New, M.B. 2002. *Farming of Freshwater Prawns. A manual for the culture of the giant river prawn (Macrobrachium rosenbergii).* FAO, Rome, 212 pp.

Nichols, J.W. 1994. Nutritive value and the bioencapsulating properties of earthworms, and their effects upon fish growth. Bachelor of Science (Honours). Flinders University: Adelaide.

Oellermann, L. and Hecht, T. 1998. The optimum density for intensive culture of a hybrid clariid catfish (*Heterobranchus longifilis* x *Clarias gariepinus*). African Fishes and Fisheries Diversity and utilisation: 228 pp.

Ogle, J.T., Nicholson, L.C., Barnes, D.N., Shields, R.J. and Lotz, J.M. 2001. Recent developments in the culture of larval red snapper *Lutjanus campechanus*. Aquaculture 2001.

Oikawa, C.K. and March, B.E.A. 1996. A method for assessment of the effectiveness of feeding stimulants for salmonid fish. *Bulletin of the Aquaculture Association of Canada, St Andrews NB.* **96**: 29-31.

Oppedal, F., Taranger, G.L., Juell, J. and Fosseidengen, J.E. 1977. Light intensity affects growth and sexual maturation of Atlantic salmon (*Salmo salar* L.) postsmolts in sea cages. *Aquatic Living Resources* **10**: 351-357.

O'Sullivan, D. and Kiley, T. 1997. Status of Australian aquaculture in 1995/96. *Austasia Aquaculture Trade Directory 97*: ii-xv.

O'Sullivan, D. and Roberts, N. 2001. Status of Australian aquaculture in 1997/98. *Austasia Aquaculture Trade Directory 1999-2000*: 3-17.

O'Sullivan, D. and Dobson, J. 2001. Status of Australian aquaculture in 1998/99. *Austasia Aquaculture Trade Directory 2001-2002*: 3-19.

O'Sullivan, D. and Savage, J. 2004. Status of Australian aquaculture in 2001/2002. *Austasia Aquaculture Trade Directory 2004*: 5-22.

O'Sullivan, D., Savage, J. and Fay, A. 2006. Status of Australian Aquaculture in 2003/2004. *Austasia Aquaculture Trade Directory 2006*: 5-23.

Otteraa, H., Hemre, G.I. and Lie, O. 1994. Influence of dietary water content on feed intake, growth and survival of juvenile Atlantic cod, *Gadus morhua* L., during the weaning process. *Aquaculture and Fisheries Management* **25** (9): 915-926.

Panagiotaki, P., Psochiou, E., Tzortzios, S. and Theodorou, A.I. 2001. Size grading in sea bream *Sparus aurata*: effect on growth and size variation. 10th Panellenic Congress of Ichthyologists, Chania, Greece, Oct. 18-20, 2001., Chania (Greece), Panellenic Ichthyological Association, Athens (Greece) pp. 221-224.

Parazo, M.M., Avila, E.M. and Reyes, D.M. 1991. Size and weight dependent cannibalism in hatchery-bred sea bass (*Lates calcarifer* Bloch). *Journal of Applied Ichthyology* **7**: 1-7.

Partridge, G.J. and Southgate, P.C. 1999. The effect of binder composition on ingestion and assimilation of microbound diets (MBD) by barramundi *Lates calcarifer* Bloch larvae. *Aquaculture Research* **30** (11-12): 879-886.

Person-Le Ruyet, J. 1990. Early weaning of marine fish larvae onto microdiets: constraints and perspectives. *AQUACOP IFREMER Actes de Colloques* **9**: 611-623.

Plumb, J.A. 1999. Overview of Warmwater Fish Diseases. *Journal of Applied Aquaculture* **9** (2): 1-10.

Pollard, D.A., Llewellyn, L.C. and Tilzey, R.D.J. 1980. Management of freshwater fish and fisheries. In: Williams, W.D. (Ed.), *An Ecological Basis for Water Resource management*. ANU Press, Canberra, pp. 227-279.

Ponpornpisit, A., Endo, A. and Murata, H. 2000. Experimental infections of a ciliate *Tetrahymena pyriformis* on ornamental fishes. *Fisheries Science* **66**: 1026-1031.

Pusey, B., Kennard, M. and Arthington, A. 2004. *Freshwater Fishes of North-Eastern Australia*. 1. CSIRO Publishing, Collingwood, 684 pp.

Pusey, B.J. and Kennard, M.J. 2001. *Guyu wujalwujalensis* new genus and species (Pisces:Percichthyidae) from north-eastern Queensland, Australia. *Ichthyological Explorations in Freshwater* **12** (1): 17-28.

Qin, J. and Fast, A.W. 1996. Size and feed dependent cannibalism with juvenile snakehead *Channa striatus*. *Aquaculture* **144** (4): 313-320.

Queiroz, J.F. and Boyd, C.E. 1998. Effects of bacterial inoculum in channel catfish ponds. *Journal of the World Aquaculture Society* **29**: 67-73.

Reynolds, L.F. 1976. Fish tagging in the River Murray. *SAFIC* **8**: 11-15.

Roberts, R.J. 1993. Motile Aeromonad Septicaemia. In: Inglis, V., Roberts, R.J. and Bromage, N.R. (Eds.), *Bacterial Disease of Fish*. Blackwell Science Ltd, Oxford, pp. 143-174.

Roncarati, A., Melotti, P., Mordenti, O. and Gennari, L. 1997. Influence of stocking density of European eel (*Anguilla anguilla*, L.) elvers on sex differentiation and zootechnical performances. *Journal of Applied Ichthyology* **13** (3): 131-136.

Rosenlund, G., Stoss, J. and Talbot, C. 1997. Co-feeding marine fish larvae with inert and live diets. . *Aquaculture* **155**: 183-191.

Rowland, S.J. 1983a. The hormone induced ovulation and spawning of the Australian freshwater fish golden perch, *Macquaria ambigua* (Richardson)(Percichthyidae). *Aquaculture* **35**: 221-238.

Rowland, S.J. 1983b. The hormone-induced spawning and larval rearing of Australian native freshwater fish, with particular reference on the golden perch, *Macquaria ambigua*. Proceedings of the First Freshwater Aquaculture Workshop, February 1983., Sydney, Department of Agriculture, New South Wales.

Rowland, S.J., Dirou, J. and Selosse, P. 1983. Production and stocking of golden and silver perch in New South Wales. *Australian Fisheries* **42** (9): 24-28.

Rowland, S.J. 1984. The hormone induced spawning of silver perch, *Bidyanus bidyanus* (Mitchell)(Teraponidae). *Aquaculture* **42**: 83-86.

Rowland, S.J. and Ingram, B.A. 1991. Diseases of Australian native freshwater fishes with particular emphasis on the ectoparasitic and fungal diseases of Murray Cod (*Maccullochella peeli*), golden perch (*Macquaria ambigua*) and silver perch (*Bidyanus bidyanus*). Fisheries Bulletin **4**. New South Wales Fisheries: Sydney.

Rowland, S.J. and Barlow, C.G. 1991. Fish biology the right prerequisites, a case study with freshwater silver perch (*Bidyanus bidyanus*). *Austasia Aquaculture* **5** (5): 27-30.

Rowland, S.J. 1994. Preliminary evaluation of the Australian freshwater fish silver perch, *Bidyanus bidyanus*, for pond culture. *Journal of Applied Aquaculture* **4**: 39-48.

Rowland, S.J., Clark, K., Hollis, M. and Pontifex, T. 1994. Production of fingerling *Bidyanus bidyanus* (Teraponidae) at two densities in earthen ponds in Australia. *The Progressive Fish-Culturist* **56**: 296-298.

Rowland, S.J. and Bryant, C. 1994. Silver Perch Culture. Proceedings of Silver Perch Aquaculture Workshops. Silver Perch Aquaculture Workshops, Grafton, Austasia Aquaculture, Sandy Bay, Tasmania, Australia.

Rowland, S.J. 1995a. Aspects of the reproductive biology and hatchery production of the Murray Cod, golden perch and silver perch from the Murray-darling River system. *Fisheries Management Paper, Fisheries Department of Western Australia.* **83**: 38-49.

Rowland, S.J. 1995b. High density pond culture of silver perch, *Bidyanus bidyanus*. *Asian Fisheries Science* **8**: 73-79.

Rowland, S.J., Allan, G.L., Hollis, M. and Pontifex, T. 1995. Production of the Australian freshwater silver perch, *Bidyanus bidyanus* (Mitchell) at two densities in earthen ponds. *Aquaculture* **130**: 317-328.

Rowland, S.J. and Walker, T. 1995. Stunting highlights need for good husbandry of silver perch. *Austasia Aquaculture* **9**: 3-4.

Rowland, S.J. 1996. Development of techniques for the large scale rearing of larvae of the Australian freshwater fish golden perch, *Macquaria ambigua* (Richardson, 1845). *Marine and Freshwater Research* **47**: 233-242.

Rowland, S.J., Read, P., Glendenning, D. and Allan, G.L. 2001. Guidelines for feeding silver perch: 4 pp.

Rowland, S.J., Allan, G.L., Hollis, M. and Pontifex, T. 2004. Production of silver perch (*Bidyanus bidyanus*) fingerlings at three stocking densities in cages and tanks. *Aquaculture* **229**: 193-202.

Rubio, V.C., Sánchez-Vázquez, F.J. and Madrid, J.A. 2003. Nocturnal feeding reduces sea bass (*Dicentrachus labrax*, L) pellet - catching ability. *Aquaculture* **220** (1-4): 697 - 705.

Ruello, N. 2000. Report on Marketing Trials on Walkamin Farmed Golden Perch. Ruello and Associates Pty Ltd.: Sydney. 19 pp.

Saillant, E., Fostier, A., Menu, B. and Chatain, B. 2001. Sexual growth dimorphism in the European sea bass (*Dicentrarchus labrax*). *Aquaculture* **202**: 371-387.

Saillant, E., Fostier, A., Haffray, P., Menu, B., Laureau, S., Thimonier, J. and Chatain, B. 2003. Effects of rearing density, size grading and parental factors on sex ratios of the sea bass (*Dicentrarchus labrax* L.) in intensive aquaculture. *Aquaculture* **221** (1-4): 183-206.

Schmitz, O., Greuel, E. and Pfeffer, E. 1982. Studies of digestion and utilization of organic nutrients by growing eels. *Zeitschrift für Tierernährung und Futtermittelkunde* **48** (3): 138-142.

Shariff, M., Yusoff, F.M., Devaraja, T.N. and Rao, P.S.S. 2001. The effectiveness of a commercial microbial product in poorly prepared tiger shrimp, *Penaeus monodon* (Fabricus) ponds. *Aquaculture Research* **32**: 81-87.

Sheikh-Eldin, M., De Silva, S.S. and Ingram, B.A. 1997. Effects of diets and feeding rate on the survival and growth of Macquarie perch (*Macquaria australasica*) larvae, a threatened Australian native fish. *Aquaculture* **157**: 35-50.

Shoemaker, C.A., Klesius, P.H., Lim, C. and Yildirim, M. 2003. Feed deprivation of channel catfish, *Ictalurus punctatus* (Rafinesque), influences organosomatic indices, chemical composition and susceptibility to *Flavobacterium columnare*. *Journal of Fish Diseases* **26**: 553-561.

Silverstein, J.T. and Freeman, D.W. 2001. Feed intake of large and small catfish in mixed size populations. Aquaculture 2001, Lake Buena Vista, Florida, World Aquaculture Society pp. 593.

Sissins, B. 2004. Lake Eppalock low water tactics. *Freshwater Fishing* **66**: 16-20.

Sloan, M.D. 1986. The effects of protein level on growth, food conversion efficiency, protein digestibility and body composition of juvenile golden perch. B. App. Sc. Queensland Institute of Technology: Brisbane.

Soltani, M. and Burke, C.M. 1994. Responses of fish-pathogenic *Cytophaga / Flexibacter*-like bacteria (CFLB) to environmental conditions. *Bulletin of the European Association of Fish Pathologists* **14**: 185-187.

Soltani, M., Munday, B.L. and Burke, C.M. 1996. The relative susceptibility of fish to infections by *Flexibacter columnaris* and *Flexibacter maritimus*. *Aquaculture* **140**: 259-264.

Stevenson, W. and Grant, E.M. 1957. Experiments upon impounded callop or yellowbelly (*Plectroplites ambiguus* (Richardson)) at Somerset Dam. *Department of Harbours and Marine. Ichthyological Notes.* **1** (3): 72-116.

Stewart, A.B., Spicer, A.V., Inskeep, E.K. and Dailey, R.A. 2001. Steroid hormone enrichment of *Artemia* nauplii. *Aquaculture* **202** (1-2): 177-181.

Stewart, A.J. and Loar, J.M. 1993. Spatial and Temporal Variation in Biomonitoring Data. In: Loeb, S.L. and Spacie, A. (Eds.), *Biological Monitoring of Aquatic Ecosystems.* Lewis Publishers, Boca Raton, USA.

Stickney, R.R. 1986. *Culture of Non-Salmonid Fishes.* CRC Press, Boca Raton, Florida.

Strauss, R.E. and Bond, C.E. 1990. Taxonomic Methods: Morphology. In: Schreck, C.B. and Moyle, P.B. (Eds.), *Methods for Fish Biology*. American Fisheries Society, Bethesda, Maryland, USA, pp. 109-140.

Strussmann, C.A., Takashima, F. and Toda, K. 1996. Sex differentiation and hormonal feminization in pejerrey *Odontesthes bonariensis*. *Aquaculture* **139** (1-2): 31-45.

Sunde, L.M., Imsland, A.K., Folkvord, A. and Stefansson, S.O. 1998. Effects of size grading on growth and survival of juvenile turbot at two temperatures. *Aquaculture International* **6** (1): 19-32.

Tamazouzt, L., Chatain, B. and Fontaine, P. 2000. Tank wall colour and light level affect growth and survival of Eurasian perch larvae (*Perca fluviatilis* L.). *Aquaculture* **182**: 85 - 90.

Tay, S.H. and Seow, P.C. 1974. Observations on the monoculture of induced bred *Oxyeleotris marmorata*, Bleeker (Marble goby) in a pond at Sembawang. *Singapore Journal of Primary Industries* **2**: 150-154.

Thune, R.L., Stanley, L.A. and Cooper, R.K. 1993. Pathogenesis of Gram-negative bacterial infections in warmwater fish. *Annual Review of Fish Diseases* **3**: 37-68.

Tikel, D., Ovenden, J. and Street, R. 2002. Genetic assignment of golden perch (*Macquaria ambigua*). Murray-Darling Basin (MDB) Fish Rehabilitation Project. Department of Primary Industries, Queensland: 12 pp.

Toranzo, A.E., Baya, A.M., Robertson, B.S., Barja, J.L., Grimes, D.J. and Hetrick, F.M. 1987. Specificity of slide agglutination test for detecting bacterial fish pathogens. *Aquaculture* **61**: 81-97.

Trippel, E.A. and Neil, S.R.E. 2003. Effects of photoperiod and light intensity growth and activity of juvenile haddock (*Melanogrammus aeglefinus*). *Aquaculture* **217** (1-4): 633 - 645.

Tucker, J.W. 2001. Barramundi culture. Aquaculture 2001, World Aquaculture Society pp. 652.

Tucker, J.W., Russell, D.J. and Rimmer, M.A. 2002. Barramundi culture: A success story for aquaculture in Asia and Australia. *World Aquaculture* **33** (3): 53-59,72.

Verschuere, L., Rombaut, G., Sorgeloos, P. and Verstraete, W. 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiology and Molecular Biology Reviews* **Dec 2000**: 655-671.

Wakabayashi, H. 1991. Effect of environmental conditions on the infectivity of *Flexibacter columnaris* to fish. *Journal of Fish Diseases* **14** (3): 279-290.

Wakita, K., Imai, S. and Hatai, K. 2002. Histopathological study on *Tetrahymena* infection in Dwarf Gourami (*Colisa lalia*). 5th Symposium on Diseases in Asian Aquaculture 24-28 Nov 2002., Gold Coast, Australia.

Walford, J., Lim, T.M. and Lam, T.J. 1991. Replacing live foods with microencapsulated diets in the rearing of seabass (*Lates calcarifer*) larvae: Do the larvae ingest and digest protein-membrane microcapsules?. *Aquaculture* **92** (2-3): 225-235.

Walford, J. and Lam, T.J. 1991. Growth and survival of seabass (*Lates calcarifer*) larvae fed microencapsulated diets alone or together with live food. Larvi'91., Special Publication, European Aquaculture Society.

Wallace, J.C. and Kolbeinshavn, A.G. 1988. The effect of size grading on subsequent growth in fingerling Arctic charr, *Salvelinus alpinus* (L.). *Aquaculture* **73** (1-4): 97-100.

Watson, C. 2001. Dollar-for-dollar native fish stocking program. *Fisheries N.S.W.* **4** (1): 36-37.

Williams, K. and Barlow, C.G. 1993. Dietary requirements and optimal feeding practices for barramundi (*Lates calcarifer*). Fisheries Research and Development Corporation, 1992/063 (93/063): Brisbane.

Williams, K.C., Barlow, C.G., Rodgers, L.J. and Ruscoe, I.M. 2003. Potential of meat meal to replace fish meal in extruded dry diets for barramundi *Lates calcarifer* (Bloch). I. Growth performance. *Aquaculture Research* **34**: 23-32.

Willis, D.W. and Flickinger, S.A. 1981. Intensive culture of largemouth bass fry. *Transactions of the American Fisheries Society* **110** (5): 650-655.

Wohlfarth, G.W. and Moav, R. 1994. Genetic testing of common carp in cages. 3. Comparison of the effect of grading and multiple nursing on growth. *Aquaculture* **127**: 115-129.

Wright, J.F. 1981. *Tetrahymena pyriformis* (Ehrenberg) and *T. corlissi* Thompson parasitic in stream dwelling triclads (Platyhelminthes: Turbellaria). *Journal of Parasitology* **67**: 131-133.

Zakes, Z. 1997. Converting pond-reared pikeperch fingerlings, *Stizostedion lucioperca* (L.), to artificial food-effect of water temperature. *Archives of Polish Fisheries* **5** (2): 313-324.

Zhang, J., Zhou, G., Li, C. and Xi, B. 2002. The development and application of feed attractants in aquatic feeding. *Journal of the Ocean University of Qingdao* **32**: 272-276.

Zitzow, R.E. and Millard, J.L. 1988. Survival and growth of lake whitefish (*Coregonus clupeaformis*) larvae fed only formulated dry diets. *Aquaculture* **69**: 105-113.

Appendices

Appendix 1 Animal Ethics Certifications.

Animal ethics clearance was sought for all experiments. The Local Animal Ethics Group for the Cairns region was responsible for assessing, recommending revisions, and approving all experimentation. Approval numbers for experiments in each chapter are listed below. Most approvals were for multiple experiments, as techniques and treatment of animals was essentially the same.

Chapter 1 and 2 – FNQ–02–01

Chapter 2 – FNQ–09–01

Chapter 3 – FNQ–01–01, FNQ–02–01,FNQ–08–01

Chapter 4 – FNQ–01–01, FNQ –06-01, FNQ-08-01

Chapters 5,6,7 –FNQ–01–01, FNQ –06-01, FNQ–08–01

Appendix 2 Publications arising from this thesis.

Herbert, B.W. and Graham, P.A. 2003. Use of *Artemia* Frozen Zooplankton and Artificial Food for Weaning Fingerlings of the Freshwater Fish Golden Perch *Macquaria ambigua ambigua* (Percichthyidae). *Asian Fisheries Science* 16(1): 85-90.

Herbert, B. and Graham, P. 2004. Weaning of the golden perch, *Macquaria ambigua ambigua*, Percicthyidae, onto prepared diets. *Journal of Applied Aquaculture* 15(3/4):163-171

Herbert, B. and Graham, P. in press Nursery production of golden perch, *Macquaria ambigua,* at two densities in ponds.*Journal of Applied Aquaculture* **19**(2)