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Developing jungle perch fingerling production to improve fishing opportunities

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Abbreviations

AAQ:	Aquaculture Association of Queensland
ANSA:	Australian National Sportfishing Association
BIRC:	Bribie Island Research Centre
DAF:	Department of Agriculture and Fisheries (Queensland)
DSITI:	Department of Science, Information Technology and Innovation (Queensland)
EHMP:	Ecosystem Health Monitoring Program
EPBC:	Environmental Protection and Biodiversity Conservation Act.
FA:	Fatty acids
FAA:	Free amino acids
FFSAQ:	Freshwater Fishing and Stocking Association of Queensland
FRDC:	Fisheries Research and Development Corporation
HUFA:	Highly unsaturated fatty acid
JCU:	James Cook University
LSD:	Least significant differences
MWR:	Mackay-Whitsunday Region
PPT:	Parts per thousand
PUFA:	Polyunsaturated fatty acid
SEM:	Standard error of the mean
SEQ:	South-east Queensland
SFA:	Saturated fatty acid

Executive Summary

Researchers based at the Bribie Island Research Centre (BIRC) developed methods to produce the first ever captive reared jungle perch fingerlings. This led to the development of a hatchery manual for jungle perch production. In late 2014 and in 2015 researchers were able to make the first ever releases of jungle perch fingerlings back into rivers and streams within their historical range.

Background

Jungle perch *Kuhlia rupestris* once occurred in east coastal rivers and streams from near Cape York in far northern Queensland, south to the Richmond River in northern New South Wales. From the Mackay-Whitsunday region southwards, jungle perch populations have declined severely since the early 1960s. They are extinct in many southern catchments and just a few small remnant populations remain in the southern half of their historical distribution. Jungle perch migrate to seawater to spawn and juveniles enter freshwater at 18-20 mm in length. Adults return to freshwater after spawning. Barriers low down in the catchments disrupt this lifecycle and have led to local extinctions. Loss of riparian vegetation and altered flow regimes are also thought to have contributed to the decline of jungle perch populations.

The removal of some barriers and the construction of fishways on weirs and road crossings since the 1990s have addressed some of the factors that caused the loss of jungle perch from a number of catchments. In some catchments, landholders, local government bodies and catchment groups have also been re-establishing and protecting riparian vegetation. Therefore some catchments have become suitable for self-sustaining jungle perch populations again. However, populations of jungle perch in southern areas had become so limited that little or no natural recolonisation of these systems occurred.

Pilot work had successfully spawned jungle perch between 2002 and 2010, but no jungle perch larvae had survived beyond five days post hatch. Angling groups including FFSAQ, ANSA and Sunfish had considered the reintroduction of jungle perch back into their historical range as a high research priority. Therefore a project was commenced to solve captive breeding of jungle perch with the objective of reintroducing this species back into parts of its former range. If jungle perch could be successfully bred, then they could also potentially become part of impoundment put and take fisheries at some stage in the future, should they ever become a permitted species for stocking.

Aims and objectives

The key aims and objectives of this project were as follows

1. Develop hatchery production techniques for jungle perch fingerlings
2. Successfully release jungle perch fingerlings into suitable south-east Queensland and Mackay-Whitsunday regional waterways.
3. Communicate with anglers on the restoration of jungle perch fisheries.
4. Understand environmental factors influencing post-release survival of jungle perch in rivers.
5. Develop a jungle perch production manual for fish hatcheries.

Methodology

Broodstock jungle perch from streams in the Townsville region, the O'Connell River and from Fraser Island were held at BIRC. Broodstock were spawned by hormonal induction with Ovaprim (Syndel Laboratories Ltd).

A series of experiments were run to optimise egg incubation, egg quality and larval rearing. Broodstock were reared on two separate diets, one diet contained mostly marine components (fish, prawns, mussels, and commercial barramundi pellets) and the other diet contained a high proportion of terrestrial insects, including mealworms, giant mealworms, black soldier fly larvae and blow fly maggots. The quality of eggs and spawning events was compared between the two broodstock groups.

The morphology and biochemical composition of eggs from the two diet treatments were also compared, together with the relationships between egg biochemistry and hatch rate and egg morphology and hatch rate. The development of eggs was monitored from fertilisation to hatch and the effect of salinity on buoyancy and hatch rates of eggs was also tested.

A series of experiments were run to aid development of rearing techniques for larvae. Factors examined included, larval feeds (copepod nauplii, super small strain rotifers and oyster trochophores), rearing salinities, rearing temperatures, lighting levels, tank background colours, tank size and tank aeration. Experiments were run in 3 L larval bowls and in tanks ranging from 1.1 m to 3.1 m in diameter (1000 L to 7000 L). The effects of these variables on larval survival and swim-bladder inflation were investigated. Data were investigated by balanced ANOVA or generalised linear models of binomial proportions, followed by post hoc LSD pairwise tests.

A series of rearing experiments were also run in fertilised seawater ponds, ranging in area from 225 m^2 to 5000 m^2 and depth of 1.8 - 2.0 m. The physicochemical characteristics of the ponds and composition of pond plankton were monitored and evaluated in a multifactorial regression to determine how prevailing pond conditions affect production of fingerlings. If larvae were still present in ponds after three weeks, some supplementary feeds, including brine shrimp (*Artemia salina*) nauplii and commercial weaning diets were provided until harvest time.

Some of the fingerlings produced were experimentally released into Currumbin Creek and the Mooloolah River in south-eastern Queensland and into St Helens Creek north of Mackay. Prior to stocking, fingerlings were conditioned to predators and live feeds. Fingerlings were also micro-tagged prior to release to enable identification of stocked fish. Stocked fingerlings were monitored using various sampling techniques, including boat and backpack electrofishing; observations from the riverbank, snorkelling surveys and underwater video.

Results/key findings

Broodstock reared on the diet containing a greater proportion of marine components tended to produce better quality eggs, with higher fertilisation and hatch rates. Larger eggs tended to have better hatch rates and eggs with higher levels of the fatty acid EPA also had better hatch rates. Salinities less than 32 ppt were detrimental to egg buoyancy and hatch rates. At 28 ºC jungle perch larvae hatch 15.16 hours after fertilisation.

Jungle perch larvae reared in tanks only survived if the nauplii of the copepod *Parvocalanus crassirostris* were offered as a first feed. There was no evidence of consumption of any other live feeds in tanks. Survival was also significantly higher at full seawater salinities greater than 32 ppt. Survival was improved by addition of over-tank lighting and through use of gentle aeration. Swim-bladder inflation was also improved by addition of over-tank lighting. Higher densities of copepod nauplii also appeared to produce higher swim-bladder inflation rates. Jungle perch were difficult to wean from copepod nauplii and copepods in the first few weeks after hatch. Cultured *P. crassirostris* have a 9-10 day lifecycle and consume a lot of time, space and labour to produce in sufficient quantities for long term rearing of fish larvae.

A more cost effective option was to rear jungle perch larvae in seawater ponds with natural copepod blooms. Pond blooms could also be seeded with captive reared copepods. Stepwise multiple linear regression identified duration of copepod nauplii blooms at densities above 50 L^{-1} as a critical variable in explaining successful pond rearing of larvae through to jungle perch fingerlings. Densities of copepod nauplii must be at least 130 L^{-1} at time of first feed if jungle perch larvae are to survive. From three weeks post hatch jungle perch larvae could be successfully be weaned onto *Artemia* and commercial weaning diets.

Just over 3300 fingerlings were released into three streams. Size at release ranged from just under 50 mm to 150 mm. Fingerlings were detected post release in all streams. The majority of fingerlings detected post stocking had moved upstream from their release points, with some fish moving more

than 10 km. Fish that were detected after a month or more at large all showed evidence of growth. Some fingerlings in St Helens Creek reached almost 250 mm in length after 10 months at large. Fingerlings tended to cluster into groups in small areas of habitat and were difficult to detect. Following a heavy rain event in May 2015, major flooding occurred in both the Mooloolah River and Currumbin Creek. No fingerlings were detected after the flood. The flood may have adversely impacted the fingerlings or enabled them to disperse to parts of the catchments we were unable to sample.

Ideally stocking should continue at the three reintroduction sites, from varying parental stock, for a further three to five years to build up genetic diversity and base population sizes. Stocking over several years can also reduce the risk of certain environmental events such as floods or droughts impacting negatively on the restocking. Monitoring should also continue over the restocking period. If new recruits are detected entering the restocked systems after stocking has ceased, then the reintroductions can be deemed a success.

Implications for relevant stakeholders

This project has for the first time demonstrated the feasibility of hatchery production of jungle perch fingerlings. The research on jungle perch production has enabled a hatchery production manual with accompanying videos to be produced. This has given private commercial hatcheries the information needed to produce jungle perch fingerlings. Several hatcheries have already indicated an interest in producing jungle perch and will be assisted to do so in 2016. Currently jungle perch are not a permitted stocking species, so cannot be sold to fish stocking groups. However, hatcheries will be able to sell fingerlings to the aquarium trade or supply grow out facilities that could produce jungle perch for human consumption. Should jungle perch become a permitted species for stocking, this will provide hatcheries with a major new product option to sell to fish stocking groups. It would also benefit anglers by providing another iconic species for impoundment stocking programs. This could have flow-on benefits to regional economies through angler tourism.

Should the pilot reintroductions of jungle perch into streams result in self-sustaining jungle perch populations, then there will be three restored jungle perch populations close to major population centres. This will create a new opportunity for anglers not normally able to target jungle perch. Since the majority of anglers who target jungle perch are catch and release fishers, angling is expected to have minimal impact on recovery of the populations.

Recommendations

- The current trial reintroduction sites should be stocked for at least three to five more years. After stocking ceases the sites should be monitored for natural recruitment. Restocking streams in the historical range should only be expanded to other streams if it is demonstrated that restocking can lead to naturally recruiting populations.
- Further improvements to spawning could be made by focusing on male contribution to spawning.
- Further work could be done to optimise tank rearing of jungle perch larvae focusing on transition from copepods to other feeds.
- Further experiments on the attraction of small fingerlings to jungle perch odour, as occurs in some other catadromous species should be run. If such an attraction exists then natural recruitment back into stocked systems may be more likely.
- The potential of jungle perch as an aquaculture species should be evaluated.
- Should jungle perch become a permitted stocking species, impoundment stocking trials should be commenced. If jungle perch are a successful put and take species in impoundments this could have benefits for tourism, recreational fishers and the hatchery industry. Currently silver perch and golden perch, which are not native to east coastal catchments, are stocked into

coastal impoundments in Queensland. It is prefereable to stock an indigenous species like jungle perch, rather than translocated species.

- Southern stocks of jungle perch should be nominated for EPBC listing as vulnerable or endangered. A 50% range reduction means these fish are eligible for nomination. If the nomination is accepted, it will necessitate the development of recovery plans, which may expedite recovery of these populations. Dependent on outcomes of reintroduction trials, conservation stocking could become a part of recovery actions.

Keywords

Jungle perch, *Kuhlia rupestris*, hatchery production, restoration, fish stocking, captive breeding, larval culture, recreational fishing

Introduction

Jungle perch *Kuhlia rupestris* has a wide Indo-Pacific distribution (Feutry *et al*. 2013), but within Australia are confined to the north-east coastal region from the Richmond River in New South Wales (NSW) to Cape York in Queensland (Hutchison *et al.* 2002). The jungle perch is a popular angling species (Allen *et al.* 2002), but it has declined in the southern part of its range in Queensland and in northern NSW (Hutchison *et al.* 2002). This project was developed in response to concerns from recreational fishers about the decline in jungle perch stocks over the past decades in south-east Queensland (SEQ) and in the Mackay-Whitsunday region (MWR). Anglers have been lobbying for an effort to return jungle perch fisheries to these regions since the1990s. Restoring jungle perch fisheries has been a top priority for the Freshwater Fishing and Stocking Association of Queensland (FFSAQ) for the last decade. In early 2011, Sunfish (the peak body representing anglers in Queensland) nominated jungle perch as their top research priority and the Queensland branch of Australian National Sportfishing Association (ANSA) also indicated that restoring jungle perch fisheries was a high priority. The Queensland Fisheries Research Advisory Board (QFRAB) also nominated jungle perch as a research priority in 2011.

Jungle perch are a catadromous species. Adults reside in freshwater but migrate to breed in seawater then return to their freshwater habitats. Juveniles enter freshwater after a marine larval and early fry stage (Hogan & Nicholson, 1987; Lewis & Hogan 1987; Hutchison 2002; Henderson 2010). Past research has indicated that the decline of jungle perch was primarily due to barriers to migration built in past decades (Hutchison *et al.* 2002) disrupting spawning migrations and juvenile migrations back into freshwater, leading to whole of catchment extinctions. Reduced flows and loss of riparian habitat have probably compounded the problem (Hutchison *et al.* 2002). In recent years fish passage has been restored in several river systems, but very little or no natural re-establishment of jungle perch has been observed away from the remaining small core remnant populations. Work by catchment groups has restored or protected riparian habitats in some river systems, which together with restored fish passage, has created suitable areas for jungle perch to re-establish. Other rivers with restored fish passage have abundant areas of suitable habitat for jungle perch in which to re-establish. Modelling by Feutry *et al*. (2013) suggested that southern Queensland populations of jungle perch would be almost totally reliant on localised recruitment and indicated there was little prospect of southern populations being colonised by larval drift from the more abundant northern populations.

Therefore despite suitable habitat and fish passage rehabilitation works it appears unlikely that jungle perch would be able to re-establish fisheries in the southern part of their range without active intervention. Fishway construction and habitat restoration have provided an opportunity for re-establishment of self-sustaining populations of jungle perch into a number of suitable rivers and streams in SEQ and the Mackay Whitsunday region. However, re-establishment would need to rely mainly on fingerlings being produced or translocated for re-stocking.

Re-establishment of jungle perch populations would be an important step towards restoring riverine ecosystems. Preliminary research on past distributions and captive breeding of jungle perch by Hutchison *et al.* (2002) and later work funded by the Queensland Lifestyle Program on the breeding biology and population dynamics of jungle perch between 2007 and 2010 (Department of Agriculture and Fisheries (DAF) unpublished data; Henderson 2010) was completed in response to FFSAQ's wish to re-establish jungle perch populations in their former range. This work progressed knowledge and eventually resulted in production of larvae and an understanding of the duration of the larval marine phase (Henderson, 2010; Hamer *et al*. 2015). Subsequent work by Feutry *et al*. (2012) and Feutry *et al*. (2013) also improved knowledge of the duration of the marine larval phase. Despite improving knowledge, captive larval survival was problematic and no fingerlings were produced. At hatch jungle perch larvae are small 1.6-2.3 mm (generally less than 2.1 mm) compared to species like Australian bass and barramundi. Attempts to rear jungle perch on super small (ss) strain rotifers were unsuccessful. Survival beyond five days post-hatch at 26 ºC was unable to be achieved. It appeared larvae were not feeding in captivity and starving to death.

In March 2011 a meeting was held involving Dr Michael Hutchison and Dr Peter Lee (DAF), Barry Pollock of Sunfish, Les Kowitz of FFSAQ and Luc Van Opdenbosch of ANSA to discuss developing an FRDC research proposal. At this meeting it was agreed that the highest priority was to focus on solving production of jungle perch fingerlings. This would enable re-establishment of jungle perch fisheries in selected SEQ and MWR rivers, thereby increasing angler access to this species. It was also recognised that it was important to keep anglers informed of progress and developments and to pass on production methods to the fish hatchery industry.

Transferring production to the hatchery industry was expected to lead to opportunities to add jungle perch to coastal impoundment stocking programs in the future, should it ever become a permitted species for stocking; thereby further increasing access to this species by anglers. The Aquaculture Association of Queensland (AAQ) had expressed an interest in jungle perch production and some members had indicated an interest in producing jungle perch, particularly if it was added to the impoundment stocking program. There was also some interest in producing this species for the aquarium trade. Therefore it was considered that an important outcome of this research should also be the production of a jungle perch hatchery manual to facilitate industry uptake of jungle perch production.

This project addressed a number of FRDC strategic challenges or research themes. The project results will primarily benefit the recreational sector, with additional benefits to the commercial hatchery sector that supports stocked recreational fisheries. Reintroducing jungle perch into restored river systems to develop self-sustaining populations supports ecologically sustainable development of fisheries as well as restoring ecosystem health and function. Development of production methods for jungle perch fingerlings will create a potential new product for the hatchery sector, not only for stocking programs, but potentially also for the aquarium trade or possibly grow out for aquaculture. This is linked to the FRDC theme of production growth and profitability.

In summary, jungle perch once occurred widely in coastal Queensland rivers from Cape York to the Gold Coast and also into northern NSW (Hutchison *et al*. 2002). Unlike barramundi, mullet and bass, jungle perch adults cannot persist long-term in saltwater habitats. Riverine barriers have led to rapid local extinction. Construction of fishways on barriers in the past 15 years has created suitable conditions for the reintroduction of jungle perch. There are limited opportunities for natural recolonisation, which has been extremely limited or non-existent into any of the restored rivers.

Translocation of adult fish is one strategy that has been used to re-establish populations of threatened fish (Todd & Lintermans 2015). Moving adults has the advantage of fish being already well adapted to the wild and therefore likely to have high levels of survival. However taking enough adults to establish sufficiently genetically diverse populations can have negative impacts on the viability of the donor populations (Todd & Lintermans 2015). Stocking of wild caught juveniles is another option. Todd and Lintermans (2015) estimated that 600 wild juvenile females (and males) would need to be translocated each year for five years to successfully re-establish populations of Macquarie perch *Macquaria australasica* at new sites in the Australian Capital Territory (ACT.) Remnant adult populations of jungle perch in south-east Queensland and the Mackay Whitsunday Region are too few for translocation of wild adults to be a practical solution without impacting on the remnant populations themselves. So as not to impact on viable remnant populations, during pilot work efforts were made to capture large numbers of wild fingerlings below barriers to migration in short streams on Fraser Island but no fingerlings were captured at these locations. The numbers of juvenile jungle perch observed in Eli Creek and Wyuna Creek on Fraser Island (the largest remnant populations in SEQ) were similar to the numbers of adults observed and it was feared taking sufficient juveniles for translocation over a number of years could adversely affect the viability of those remnant populations. Restocking from captive bred individuals appeared to be the only viable option to restore jungle perch fisheries to their historical range. On-growing captured juveniles and sub-adults from remnant populations for use as broodstock was believed to be the lowest impact option for the remnant donor populations.

Restoring wild jungle perch fisheries is a high priority for recreational fishers in Queensland. Reintroduction of self-sustaining populations in rivers in south-east Queensland, the Mackay-

Whitsunday Region and possibly northern NSW will provide angling opportunities to a large population of anglers.

Should jungle perch become a permitted species for stocking, development of jungle perch fingerling production will also create future opportunities to further enhance Queensland's stocked impoundment fisheries and make jungle perch accessible to even more anglers. Research by DAF (2002-2010) solved much of the reproductive biology of jungle perch, to a point where they could be spawned regularly in captivity. The transition from larvae to fingerlings is critical for future development of jungle perch fisheries and has been the main focus of this research project. Determining stocking success of fingerlings produced is also important and will help guide reintroduction of jungle perch fingerlings into the future.

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Objectives

The following are the broad objectives for the whole project:

1. Develop hatchery production techniques for jungle perch fingerlings
2. Successfully release jungle perch fingerlings into suitable south-east Queensland and Mackay-Whitsunday regional waterways.
3. Communicate with anglers on the restoration of jungle perch fisheries.
4. Understand environmental factors influencing post-release survival of jungle perch in rivers.
5. Develop a jungle perch production manual for fish hatcheries.

More specific objectives to achieve each of the above broad objectives will be outlined in the chapters and appendices of this document

Chapter 1: Spawning, egg management, egg quality and embryological development

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Summary

Jungle perch *Kuhlia rupestris* were induced to spawn using an intraperitoneal injection of Ovaprim solution (Syndel laboratories Ltd) at the rate of 1 mL.kg^{-1} bodyweight for males and 0.75 mL kg^{-1} bodyweight for females. Females over 2.5 kg body weight were given a lower dose of 0.50 mL kg^{-1}. At 28 ºC, mean latency period to spawning following hormonal induction was 55 h 21 min and the median latency period was 54 h 6 min. Fertilisation rates were not normally distributed. A large number of samples had fertilisation rates greater than 50%, but there was a peak of samples with fertilisation rates of less than 5%. The mean fertilisation rate was 56.6% and the median fertilisation rate 65%. The potential contribution of male quality to fertilisation rate was not able to be determined. Fertilisation rates had improved significantly compared to previous pilot studies. Improvements are probably related to better control of salinity and temperature during spawning.

Jungle perch produce clear spherical pelagic eggs. Fertilised eggs have a mean diameter of 660 µm (range 620-710 µm). Egg diameter was positively correlated with hatch rate ($R^2 = 0.744$, $p < 0.01$). Egg diameter is therefore a useful predictor of egg quality. The number of eggs spawned was negatively correlated with egg size across all spawning seasons ($R^2 = 0.157$, $p < 0.01$), but the number of eggs spawned was not significantly correlated with broodstock weight.

Maintaining salinity at or above 32 ppt is essential for fertilisation and maximising hatch rates. Buoyancy of fertilised eggs increase with increasing salinity. At salinities below 32 ppt it is difficult to separate fertilised eggs from dead or unfertilised eggs, which tend to sink. Separation of fertilised from unfertilised eggs is important to maintain water quality in incubation and hatch tanks.

Jungle perch eggs spawned by adults fed a diet high in marine components (including a commercial pellet diet, prawns, fish and mussels), had higher fertilisation rates than eggs spawned by adults on a diet high in terrestrial invertebrate components (including giant mealworms, meal worms, black soldier fly larvae and blow fly larvae). There were no statistically significant differences in proportions of total protein, dry matter, total lipid and free amino acids in eggs between the two diet treatments. However, there was some variation in fatty acid profiles between eggs from broodstock fed the marine or terrestrial based diets. The saturated fatty acid, palmitic acid (16:0) was the most abundant fatty acid in the eggs of jungle perch from both broodstock diet treatments. However, palmitic acid was significantly higher in eggs from the invertebrate diet group than in eggs from the standard diet group. The most abundant unsaturated fatty acids in the eggs from both the invertebrate and standard diet groups included the monounsaturated oleic acid (18:1n - 9), the polyunsaturated Linoleic acid (18:2n - 6) and Docosahexaenoic acid (22:6n - 3) (DHA).

The invertebrate diet group had eggs with significantly lower n - HUFA fatty acid proportion, and higher levels of linoleic and oleic acid than standard diet group eggs. In comparison significantly higher quantities of DHA, Eicosapentaenoic acid (20:5n - 3) (EPA) Docosapentaenoic acid (22:5n - 3) and n -3 HUFA were observed in eggs from the standard diet group compared to the invertebrate diet group.

The amount of saturated fatty acids (SFA) were negatively correlated with hatching success (p=0.034) and the amount of EPA was positively associated with hatching success (p=0.050). These results, combined with higher fertilisation rates for eggs from the marine-based diet group, suggest jungle perch broodstock fed a diet high in marine components produce higher quality eggs than broodstock fed a high proportion of terrestrial invertebrates.

Jungle perch eggs display meroblastic cell division and follow the general pattern of embryogenesis observed in other teleosts. Development at 28 ºC was rapid. Hatching commenced at 15.16 h or 424.5 degree-hours post-fertilisation (dhpf) and the entire batch of eggs was fully hatched by 15.41 hours (431.5 dhpf). Jungle perch yolk-sac larvae were sensitive to handling. To minimise mortalities was prefered to move embryos (10-14 h post-fertilisation) rather than yolk-sac larvae to larval rearing tanks or ponds.

Iodine is commonly used as a prophylactic treatment for bacteria on fish eggs. Jungle perch eggs were sensitive to disinfection treatment with iodine solution in comparison to several other marine and freshwater species. Even an iodine dose of 15 mg L^{-1} for 10 minutes (less than the standard dose of iodine applied to the eggs of many other species) resulted in significantly lower hatch rates for jungle perch than untreated controls.

Introduction

Jungle perch *Kuhlia rupestris* are part of the *Kuhliidae* family which contains twelve species found in the tropical and subtropical waters of the Pacific and Indian oceans (Randall & Randall 2001). Six species are strictly marine, one species is marginally diadromous (marine larvae and estuarine adults) and five species are strictly diadromous (Randall & Randall 2001). Four species of *Kuhlia* are known from Australian waters: *K. rupestris, K. munda, K. mugil and K. marginata*. Most of these species are not common. *Kuhlia rupestris* and *K. marginata* are the only species in the genus *Kuhlia* that enter freshwater in Australia (Randall & Randall 2001; Pusey *et al.* 2004). Jungle perch is the largest member of the *Kuhliidae* family. Merrick & Schmida (1984) reported it reaching a length of 450 mm and 3 kg in weight. Jungle perch held in captivity at BIRC have exceeded 500 mm in length and reached more than 3.5 kg in weight.

Jungle perch has only been recorded in easterly flowing drainages in north-eastern Australia, north from the Richmond River in NSW to the Cape York region (Hutchison *et al.* 2002; Pusey *et al.* 2004). Jungle perch, is a popular species for recreational angling (Hutchison *et al.* 2002; Pusey *et al*. 2004); however, jungle perch numbers have declined severely in the southern half of their historical range in Australia over the last 50-60 years. There is considerable interest among recreational fishers in restoring populations in this region (Hutchison *et al.* 2002). Barriers imposed to fish passage such as weirs and dams are thought to be largely responsible for the decline of jungle perch, with other factors such as loss of riparian vegetation and reduced flows probably also contributing to reduced numbers (Hutchison *et al*. 2002; Pusey *et al*. 2004). Recovery of populations following removal of barriers to fish movement appears to occur slowly, if at all (Hutchison *et al.* 2002), implying that recolonisation between freshwater drainages via marine habitat may not be common. Modelling by Feutry *et al.* (2013) suggested that southern Queensland populations of jungle perch would be almost totally reliant on localised recruitment and indicated there was little prospect of southern populations being colonised by larval drift from the more abundant northern populations.

At the time this project began (2012), the reproductive biology of wild jungle perch was quite poorly understood. It had been shown that sperm was immotile in freshwater but active in water with higher salinities (Hogan & Nicholson 1987; Henderson 2010). Furthermore fish from southern populations

required higher salinities than fish from north Queensland for sperm to become motile (Henderson 2010). Through analysis of otolith daily rings and otolith microchemistry, it was estimated that jungle perch larvae and fry spend 35 to 50 days in marine environments before entering freshwater (Henderson 2010; Feutry *et al.* 2012; Feutry *et al.* 2013; Hamer *et al.* 2015). These data suggested that wild jungle perch may breed in lower estuarine or near-shore marine environments and this supports the assumptions of Lewis & Hogan (1987). Jungle perch were successfully spawned in seawater at the BIRC between 2007 and 2010, but larval survival was poor, with none surviving beyond five or six days post hatch (M. Hutchison & S. Nicholson (DAF) unpublished data).

An assessment of the reproductive biology of a captive jungle perch population at BIRC undertaken between 2007 and 2010 (M. Hutchison & S. Nicholson (DAF) unpublished data) suggested jungle perch females spawn multiple times during the spawning season and individual females can produce from 200 000 to 1 million eggs per batch, depending on fish size. Egg viability for jungle perch was found to be highly variable under culture conditions (Hutchison & Nicholson (DAF) unpublished data). Jungle perch spawning between 2007 and 2010 (DAF unpublished data; Henderson, 2010) was of limited success, and fertilisation rates were generally low (mean = 6%) and induction times varied widely (45-83 hours, mean=54 hours 30 min, median=56 hours 54 minutes).

Improving egg fertilisation rates and developing a reliable method to evaluate egg quality of jungle perch at an early stage, or to select broodstock most likely to produce good quality eggs was therefore of considerable importance.

Egg quality is one of the major constraints in the production of both marine and freshwater fish species. High-quality eggs are regarded as those that lead to high levels of survivorship at fertilisation, hatching and first feeding, producing strong healthy larvae (Kjorsvik *et al.* 1990; Folkvord *et al.* 1994). Egg quality is influenced by both pre-fertilisation (broodstock management, biochemical composition, morphology) and post fertilisation factors (husbandry, physiochemical conditions). These are parameters that may change during the reproductive season (Lavens *et al.* 1999). Therefore, it is important to investigate the existence of seasonal variations in egg quality, in order to optimise egg collection and larval production.

Embryos and yolk-sac larvae develop using primarily endogenous nutrition sources (Brooks *et al.* 1997). As a consequence egg biochemical composition is a major factor in determining the quality and successful development of these stages (Brooks *et al.*1997). Various biochemical components have been identified as essential in providing embryonic and larval development in teleost fishes. Studies focussing on biochemical analyses have included measurements of yolk protein composition (Olin & Von der Decken 1990), free amino-acids (Rønnestad *et al.* 1992), lipid analysis (Sargent 1995; Salze *et al.* 2005; Johnson 2009), carbohydrate composition (Giménez *et al.* 2006), vitamin contents (Palace & Werner 2006) and enzymatic activities (Lahnsteiner *et al.* 1999). Broodstock diet has been shown to significantly influence the biochemical composition of eggs (Bell *et al.* 1997). The deposition of essential nutrients into the developing oocytes can be affected by the nutritional status of the broodstock at the time of gonad maturation and vitellogenesis (Lavens *et al.* 1999). Knowledge of the nutritional requirements of eggs and yolk sac larvae are required in order to compose a satisfactory broodstock diet to produce good quality eggs.

Other factors that can influence biochemical composition of eggs include intra-specific genetic differences (Lavens *et al.* 1999), age (Evans *et al.* 1996) and spawning period (Ma *et al.* 1998; Kamler 2005).

The role of proteins and free amino acids (FAA) in egg development has been well established in marine fish (Rønnestad *et al.* 1998; Rønnestad *et al.* 1999; Moran *et al.* 2007; Finn & Fyhn 2010). In fish, FAA are the primary metabolic energy source during the egg stage while fatty acids (FA) primarily supply energy during the yolk-sac stage (Sink *et al.* 2010). Protein and FAA have been found to be valid indicators of egg quality, although they have received less research than lipids (Washburn *et al.* 1990; Harel *et al.* 1995).

The incorporation, synthesis and processing of egg components that occur during oogenesis play a key role in the coordinated assembly of a high quality oocyte that will, once fertilised, develop into a normal embryo (Bobe & Labbe 2010). In fish, maternally derived lipids are sequestered by the oocyte during oogenesis and play two roles. They provide the embryo and developing larvae with an energy substrate for catabolism, and also essential material for the formation of cell and tissue membranes (Sargent 1995; Bell *et al.* 1997). Lipids are generally considered to be the most important energy reserve in marine fish eggs and larvae (Vetter & Hodson 1983). Lipid content influences embryonic development, survival and growth due to its critical function in membrane formation (Sargent 1995; Wiegand *et al.* 2004). Fish egg lipid varies between species and may change during different developmental stages according to the physiological events and the energy demands of the eggs (Rainuzzo *et al.* 1997).

Due to the importance of lipids in both metabolism and development, several lipid-based measures have been proposed as indicators of egg quality, mainly the essential fatty acid (EFA) profiles of docosahexaenoic acid, eicosapentaenoic acid, arachidonic acid and their ratios (Sargent 1995). Lipids play an important role as sources of EFA needed for normal growth and survival. Deficiencies in certain EFA in fish eggs can affect larval survival through behavioural changes and their ability to swim and capture food (Wiegand *et al.* 2004). The requirement by larvae of many marine fish for the highly unsaturated fatty acids (HUFA) eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid is well established. Note highly unsaturated fatty acids may be defined as fatty acids with at least 20 carbon atoms and three or more double bonds.

This chapter focuses on the initial stages of jungle perch production, including spawning induction and egg incubation and management. The project aimed to improve fertilisation rates, egg quality and egg incubation management. The effect of broodstock diet on egg quality and factors that may be indicators of egg quality were examined.

One objective of the present study was to ascertain whether the morphological egg quality parameters including egg, yolk and lipid vesicle diameter, can be reliably used to predict hatching success in captive jungle perch. Another aim was to determine if broodstock diet type, broodstock source location, broodstock type or stage of the spawning season have an impact on egg quality parameters.

Variations in the biochemical components of jungle perch eggs over the spawning season and between broodstock diets were also investigated to assess their impact on fertilisation and hatching rates. Little research has been conducted to date on the nutritional requirements of Australian freshwater aquaculture species, and no data were available on the nutritional requirements of jungle perch. Therefore, a suite of quantitative and qualitative measurements were collected to determine the broodstock dietary effects on various parameters measured for jungle perch eggs.

The effect of salinity on egg buoyancy and hatch rates was also examined with a view towards effective management of eggs during incubation. The use of iodine as a potential treatment for eggs, to prevent bacterial and other infections was also investigated. If successful, this could improve hatch rates or larval survival.

The embryological development of jungle perch was also described. This is particularly relevant in terms of when it is feasible to move embryos into larval rearing tanks or ponds prior to hatch.

Objectives

1. The work described in this chapter relates to the general objective “Develop hatchery production techniques for jungle perch fingerlings”. This is a key step toward the other general objective “Develop a jungle perch production manual for fish hatcheries”.

Spawning and egg management is a key part of hatchery production. The specific objectives in this chapter were to:

i. improve spawning success of jungle perch
ii. ascertain whether the morphological egg quality parameters, including egg, yolk and lipid vesicle diameter, can be reliably used to predict hatching success in captive *K. rupestris*
iii. determine if broodstock diet type, broodstock source location, broodstock type or stage of the spawning season have an impact on egg quality parameters
iv. quantify the biochemical composition of captive-spawned jungle perch eggs and monitor egg quality markers (hatch rate, fertilisation rate, biochemical composition) across diet treatments
v. evaluate the relationships between changes observed in biochemical composition and egg quality across diet treatments
vi. describe embryological development.
vii. optimise egg management and hatching success.

Methods

Broodstock collection

A total of 160 broodstock were held for this project. These included fish that had already been held in captivity for several years prior to this project commencing and fish collected more recently in 2012 and 2013. Long-term captive fish included some fish sourced from the Daintree catchment in far north Queensland. Daintree River fish were used to help develop production techniques but were not used for breeding for the stocking program. The majority of brood fish were collected by backpack and boat-based electrofishing. Broodstock were sourced from Fraser Island in south-east Queensland, the O'Connell River north of Proserpine, and from Rollingstone and Crystal Creeks north of Townsville. Some additional fish captured by an aquarium enthusiast from the Brunswick River in northern New South Wales were also donated to the project.

Broodstock captured in the wild were transported directly from Fraser Island by road in a purpose-built 800 L insulated fibreglass fish carrier. Fish were provided with lightly bubbled oxygen during transport to the BIRC. On arrival at BIRC the fish were placed into 7000 L or 10 000 L freshwater tanks. Fish captured near Proserpine and Townsville were transported by road in the same fish carrier to holding tanks at the Fisheries Queensland facilities in Mackay or to1000 L tanks on a recirculated freshwater system at the James Cook University Aquaculture facility in Townsville. After being held overnight, fish were then placed in large heavy duty plastic bags, one-third filled with freshwater, inflated with medical oxygen and sealed with elastic bands. The bags were then packed in polystyrene boxes, sealed with packing tape and flown directly to Brisbane. After pick up at Brisbane airport, fish were taken immediately to BIRC and placed into 7 000 L or 10 000 L freshwater tanks.

Fish from the three regions (Townsville-Proserpine, Fraser Island and nearby mainland areas, and Daintree-Cairns) were held as separate groups to maintain regional genetic integrity for the breeding program.

Broodstock management and feeding regime

Tank systems
Broodstock were maintained in 7 000 L and 10 000 L sky blue fibreglass tanks linked to a recirculated filtration system. Broodstock were held in low salinity water. Although jungle perch adults live in full freshwater, the recirculation system was maintained between 3-5 ppt salinity to reduce risk of white-spot infection. The recirculated system included a drum filter to remove solid waste, a wet and dry bio-filtration system (to remove ammonia and phosphate) and biological towers containing bio-beads. The system also contained programmable heating and chilling components to regulate temperature, and a UV system to treat the water for bacteria and other parasites.

The system also included two large variable speed drive pumps. If one pump failed the second pump would start automatically. Water was supplied to each tank at a rate of 60 $L.min^{-1}$ to ensure turnover in each tank every two hours. The system was linked to a back-up power supply in case of mains power failure.

Broodstock were held at relatively low densities, ranging from 2-5 $kg.m^{-3}$. All tanks were aerated to keep oxygen levels high and all tanks were covered with 10 mm mesh to prevent fish from jumping out of the tanks. Tanks had 7º conical bottoms to facilitate removal of waste. From October to May water temperatures were maintained close to 26 ºC (range 23.9 ºC-28.2 ºC) and for the remainder of the year close to 23 ºC (range 19.4 ºC -25.9 ºC). The broodstock recirculating system was connected to heating and chilling units, but these were still influenced to some extent by extremes of heat and cold from ambient air temperatures.

Feeding

During the breeding season and from six weeks prior to the breeding season (October to May), jungle perch broodstock were fed to satiation five days per week. In year one of the project broodstock were split into two groups. In the wild, jungle perch consume a range of terrestrial and aquatic insects, along with fish and crustaceans (Pusey *et al.* 2004). The terrestrial component of the diet of one group of broodstock was increased to determine if that had any beneficial effect on egg quality. The other group received a diet with a greater proportion of aquatic (marine) items and commercial pellets. Both groups were fed on a ten-day cycle as outlined in Table 1.1. After the first year of the project all broodstock were fed a diet containing more marine components (including commercial pellet feeds) than terrestrial components. The feeding cycle for years two and three of the project is outlined in Table 1.2. From June to September, fish were fed to satiation just three days per week (Table 1.2). The dietary components were similar to those used in the warmer months, but feeding was on a nine-day cycle in year one for the two diet groups and on a six-day cycle for the sole diet group in years two and three.

Table 1.1: Jungle perch broodstock breeding season diets in years 1, 2 and 3 of the project. * Fish generally sandy sprats or blue bait.

Day	High terrestrial insect diet year 1	High aquatic/marine diet year 1	Diet all broodstock years 2 and 3
1	prawns	prawns	Ridley barramundi pellets
2	mealworms	Ridley barramundi pellets	Prawns
3	giant mealworms	fish (pilchards or sprats)	Fish*
4	black soldier fly larvae	mussels	Ridley barramundi pellets
5	fish (pilchards or sprats)	Ridley barramundi pellets	Giant mealworms
6	Ridley barramundi pellets	Ridley barramundi pellets	Ridley barramundi pellets
7	prawns	prawns	Prawns
8	bloodworms	fish (pilchards or sprats)	Fish* and vitamins
9	blowfly maggots	Ridley barramundi pellets	Ridley barramundi pellets
10	giant mealworms	mealworms or giant mealworms	giant mealworms

Table 1.2: Winter feeding cycle in years 1, 2 and 3 of the project

Day	High terrestrial insect diet year 1	High aquatic/marine diet year 1	Di*et al* l broodstock years 2 and 3
1	prawns	prawns	Ridley barramundi pellets
2	blowfly maggots and pellets	Ridley barramundi pellets	prawns
3	mealworms	mealworms	fish
4	prawns	prawns	Ridley barramundi pellets
5	black soldier fly larvae	mussels and fish	prawns
6	giant mealworms	Ridley barramundi pellets	giant mealworms
7	fish	fish	
8	prawns	prawns	
9	giant mealworms	Ridley barramundi pellets	

Spawning induction

Prior to spawning induction jungle perch were captured from broodstock tanks following light sedation. Tank water levels were dropped to a volume of around 1000 L. AQUI-S was added to the tank at a rate of 10 mL per 1000 L to lightly sedate fish without inducing loss of equilibrium. This dose made fish slower and easier to dip net and reduced risk of stress and injury to the fish. After dip-netting individual broodstock were placed in an 80 L aerated plastic tub with a dose of AQUI-S of 1 mL per 20 L for heavy sedation. When fish lost equilibrium they were scanned for a PIT tag to identify the individual. Female fish were checked for egg condition by inserting a sterilised flexible plastic tube of 2 mm diameter into the urogenital papilla. The tube was carefully pushed down the oviduct into the ovary. Gentle sucking by mouth on the distal end of the tube was used to remove an egg sample. The collected sample was expelled onto a microscope slide and viewed under a compound microscope with the 10 x objective. A micrometer was used to estimate egg diameter. If the majority of eggs were in the size range of 380 μm to 400 μm or larger, then the fish was considered suitable for spawning induction. Suitable fish were measured to fork length and weighed. Female fish were given an injection of Ovaprim solution (Salmon GnRH 20 $\mu g.L^{-1}$ with Domperidone 10 $mg.mL^{-1}$) at the rate of 1 $mL.kg^{-1}$ bodyweight in year one of the project and for most of year two of the project. This dose was used because preliminary work during a pilot project with newly captured individuals did not achieve spawning at lower doses. However, it was found that as fish spent more time in captivity, lower doses at a rate of 0.75 mL kg^{-1} became effective. In fish larger than 2.5 kg, a dose of 0.5 $mL.kg^{-1}$ was used from year 3 to reduce risk of fish becoming eggbound. All Ovaprim injections were intra-peritoneal using a 21-gauge needle. The injection was made near the base of the pelvic fins, with the needle facing in a posterior direction to avoid any vital organs. The injection site was swabbed with iodine solution just prior to injection to reduce risk of bacterial infection.

If sedated fish were identified as male, they were gently squeezed in the abdominal region to check for running milt. Males have a smaller urogenital opening (which the cannula cannot enter) and are not as deep in the body as female fish. In most cases milt is expressed as a thin cohesive thread. More rarely, milt would run freely. If free running milt or a cohesive milt thread was detected, males were injected with a dose of 1 $mL.kg^{-1}$ Ovaprim solution following the same procedure as for females. For any given spawning up to six females were induced and males were induced at a rate of three males for every female. Four spawning tanks were generally used each run.

Fish identified as not in breeding condition were placed in a 1000 L freshwater tank to recover from sedation. After all fish from a broodstock tank had been processed, those not being used for spawning were returned to their broodstock tank.

Time of induction was recorded for each fish used for spawning. Following injection of Ovaprim solution fish were placed in 7000 L spawning tanks to recover from sedation. Spawning tanks were covered with 10 mm mesh netting to prevent fish jumping out. Tanks were well aerated. For some early spawning inductions in the first year of the project, up to three females and nine males were placed in a 7000 L spawning tank. Late in the first season this was changed to one female per tank, with three males per tank. Up to four tanks were used per spawning run. This permitted better management of genetics and more accurate identification of individual spawning events. The characteristics of spawned eggs could also be linked back to the characteristics of individual females.

Spawning tank set up and egg collection

When fish were placed into the spawning tank the salinity (3-5 ppt) and temperature (generally around 26 ºC) were the same as those in the broodstock tanks. Once the full complement of males and females were placed in a spawning tank the water level was dropped to 25% capacity. The spawning tank was then switched to a marine water recirculating system for refilling. In the 2012/13 spawning season, tanks were initially run with flow-through seawater, but after a low-salinity pulse following heavy rainfall came through the system during a spawning event, all subsequent spawns were run on a recirculating system for better salinity and temperature management. Salinity levels in the spawning tank normally rose to full seawater levels over a 12-hour period. If necessary, sea salt was added to the recirculating system to bring salinity levels up to at least 34 ppt. Water in the spawning tanks was heated to 28 ºC with 3000 W immersion heaters and by additional heating in the recirculating system. Each spawning tank had an overflow pipe that ran into a 300 μm mesh egg collecting basket set in a 200 L tub (Figure 1.1). Water was added to the spawning tanks at a rate of approximately 50 $L.min^{-1}$. Overflow water from the tub was returned to the recirculation system. Spawning generally occurred from 48 hours to 60 hours after induction (see Results section this chapter). From around 48 hours after induction, egg baskets were checked by staff at 15-minute intervals. Staff alternated checking times, such that no one staff member was required at less than 30-minute intervals.

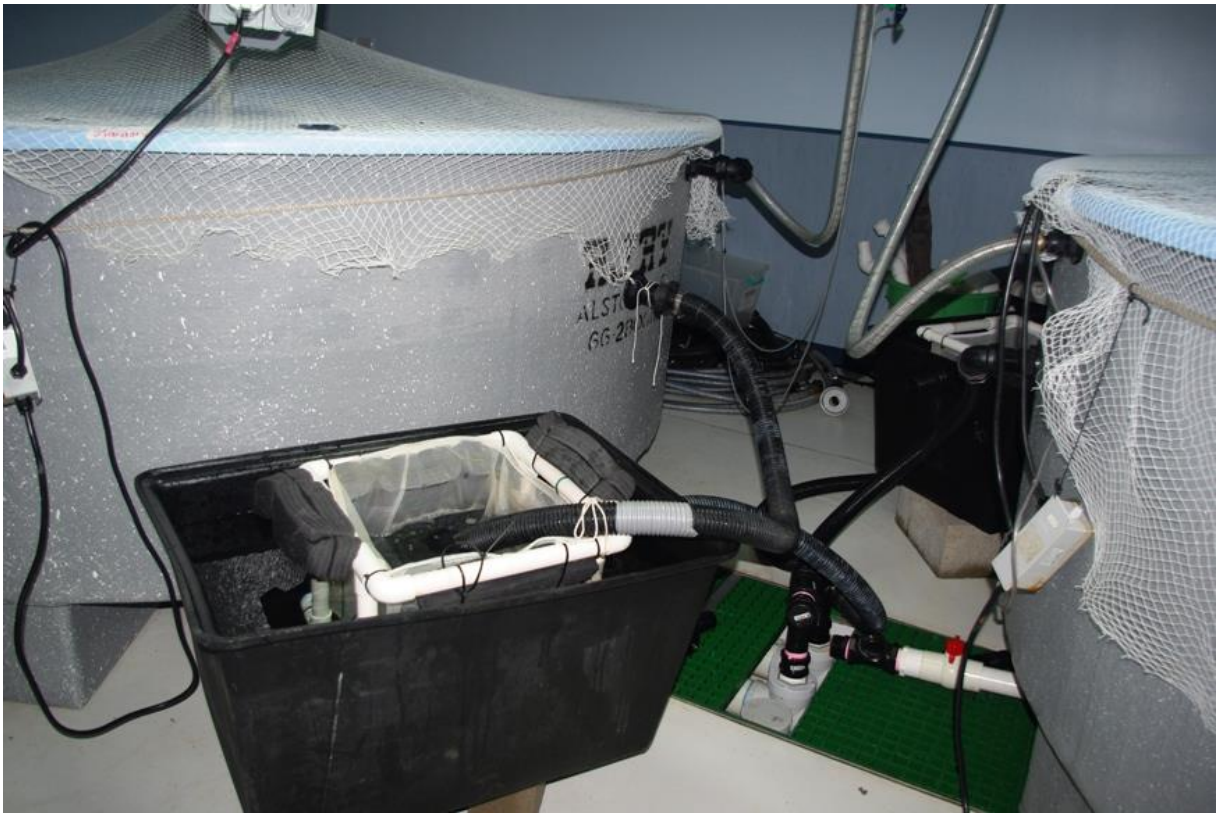

Figure 1.1: Egg collection basket in tub adjacent to spawning tank.

If eggs were found in a basket, the spawning time was recorded. Eggs were then allowed to collect in the spawning basket for at least one hour, unless being used for embryological development studies, in which case a sample was collected immediately. To collect eggs the spawning basket was partially lifted from the water to concentrate eggs in the bottom part of the basket. Eggs were scooped from the basket with plastic jugs or beakers and placed in a 20 L bucket. The bucket was filled precisely to the 20 L mark. If eggs were still visible in the spawning tank, the egg basket was set back down in the tub for more eggs to accumulate. The egg collection process would be repeated every one to two hours until almost no eggs were visible in the spawning tank.

Counting eggs and evaluating fertilisation rates

The number of eggs produced in a spawn and fertilisation rates were calculated for all spawns in this project, including spawns run for experimental purposes and spawns for production runs. Numbers of eggs in a spawn were estimated volumetrically. Eggs collected in a 20 L bucket were mixed by gentle stirring action. For low density samples (e.g. visually estimated < 2 eggs.mL^{-1}) a 10 mL sample was pipetted from the bucket and run out onto a 120 μm sieve. This was examined under a dissecting microscope and the number of eggs in the sample was counted. This process was repeated three to six times, depending on the consistency of the samples. The mean number of eggs mL^{-1} was then calculated. A 20 000 multiplier was applied to estimate the number of eggs in the bucket. If eggs were of higher density (visually estimated at 2-10 eggs.mL^{-1} or >10 eggs.mL^{-1}) the same process was used, but smaller samples of either 5 mL or 1 mL, respectively, were pipetted from the bucket. Each spawn generally consisted of several buckets of samples. Individual bucket totals were summed to produce the total spawn estimate.

Fertilisation rates were estimated in a similar fashion. After mixing the eggs in the bucket a sub-sample of eggs was pipetted from the bucket onto a petri dish to provide approximately 100 eggs for viewing under a dissecting microscope. Eggs were checked for evidence of cell division to determine fertilisation rates. In seasons 2 and 3, in most cases an equivalent sub-sample was also viewed with a Nikon Eclipse Ti inverted compound microscope using the 4x objective, set to PHL with phase

contrast. An image was captured by a Nikon digital camera mounted on the microscope and displayed on a computer screen. The diameters of eggs from experimental spawns and some production run spawns were measured in μm using a measuring tool in NIS Elements software. The measured digital images were saved by NIS Elements as .jpg files and the values obtained were used to determine a mean egg diameter for the spawn. In season 1 a different microscope system was used and its use is outlined under "Evaluating egg quality" below.

General egg management

After sub-samples of eggs had been collected from the 20 L buckets for estimates of numbers and fertilisation rates, the water in the bucket was gently swirled and then left to stand for 5-10 min. The debris (including dead eggs) that collected at the centre of the bottom of each bucket was siphoned out of the bucket with a 6 mm plastic tube. Eggs were then transferred to 1000 L hatch tanks. Hatch tanks were constructed of fibreglass and covered with a pale blue gel coat. Tanks had a 7º cone bottom. Hatch tanks were supplied with UV treated, 1 μm filtered seawater. Water was gently flowed constantly into the tanks (<5 $L.min^{-1}$) and flowed out through a central conical screen of 200 μm nylon mesh. Water level was set by an external standpipe system. Tanks were heated to 28 ºC ± 0.5º with 600 W immersion heaters. The water was gently aerated by a central air stone to create a wall of bubbles near the conical screen.

After eggs had been in the hatch tank several hours, the air, heaters and water supply were switched off temporarily, the water gently swirled and debris that accumulated in the centre of the base of the tank removed by siphon as before. Following siphoning, air, water and heat supplies were restored.

As eggs developed and progressed through the notochord stage, a noticeable darkening across the centre of the eggs became visible to the naked eye. Shortly after this stage (during the early embryo stage) the air, water and heat supplies to the hatch tanks were switched off and eggs were left to raft on the surface. Live fertilised eggs tend to raft in seawater. It takes approximately 10 to 15 min for the majority of eggs to raft. Rafting eggs were skimmed off the surface using plastic jugs or beakers (Figure 1.2). Eggs were transferred to either 80 L plastic tubs or 20 L buckets. When the buckets or tubs were full, they were gently mixed by hand and the number of eggs in each container estimated volumetrically as described above. Eggs were then distributed to various experimental tanks, ponds or containers for hatching (see Chapter 2). Preliminary work had indicated eggs were more resilient to handling and transfer than newly hatched larvae. Therefore, this stage was preferred for distribution to experimental tanks. Numbers of eggs were distributed volumetrically. For example, if 6000 eggs at embryo stage were required for hatching in an experimental tank and the egg density was 10 per mL, then 600 mL would be transferred (after mixing) from the container of eggs to the experimental tank.

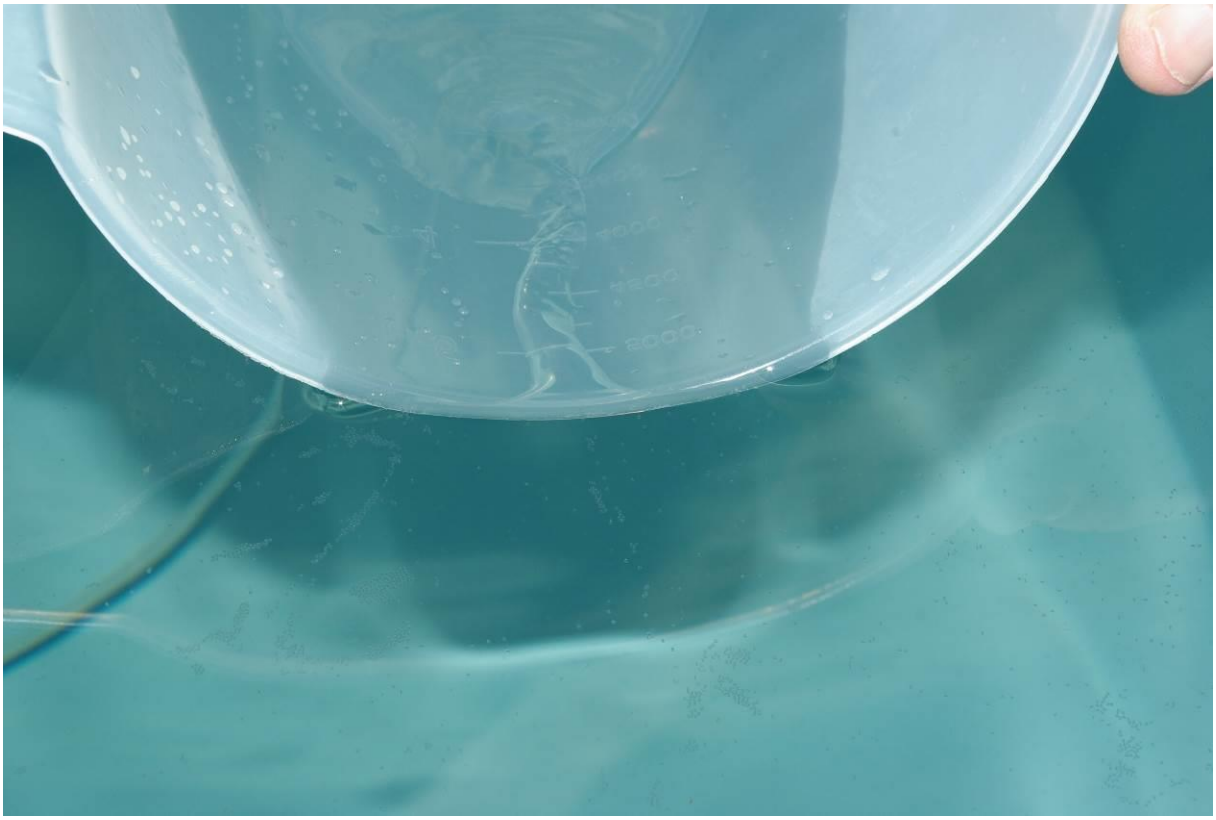

Figure 1.2: Skimming rafting eggs from the surface of a hatch tank

Not all eggs could be skimmed from the surface of the hatch tanks. The collecting process itself causes some eggs to re-enter the water column, and some live fertilised eggs are neutrally buoyant. Those eggs that could not be collected were left to hatch in the hatch tank. Water, air and heating were restored to the tank after egg collection was completed. The larvae that hatched in the tank were collected the next day. Buoyant newly hatched larvae were skimmed from the surface as was done for the eggs. The remaining larvae were collected after the tank level was dropped to less than 100 L. The central cone screen in the tank permitted lowering of the water level without loss of larvae. The remaining larvae were scooped from the water using plastic jugs and placed into 80 L plastic tubs or 20 L plastic buckets. Larval numbers were estimated volumetrically as for the eggs. These larvae were distributed volumetrically to ponds or to additional tanks for general grow out, but were not used in experimental tanks. Larvae surplus to requirements were euthanised.

Determining hatch rates

In years 1 and 2 of the project, hatch rates (the proportion of fertilised eggs in a batch that hatched) of various batches of eggs were determined using 3 L fish bowls filled with UV treated 1 µm filtered seawater to a volume of 2.5 L. Bowls were maintained in a water bath at a temperature of 28 ºC. Eggs at the embryo stage were added to the bowls volumetrically to give an estimated 300 eggs per bowl. Post hatch the bowls were gently mixed and three 50 mL subsamples were collected by submerging small 50 mL containers into the bowl. The number of yolk sac larvae in each container was counted. The mean number of larvae per sample was then calculated and multiplied by 50 to give a total estimate of the number of larvae per bowl. This was converted to a hatch rate (per cent) based on an assumed number of 300 eggs. Six to ten replicate bowls were used each spawn depending on numbers of available larvae and other experimental requirements.

Evaluating effect of salinity on egg buoyancy and hatch rate

Fertilised eggs collected from spawns in March, April and May 2013 were tested for buoyancy at 1 ppt intervals for salinities ranging from 28-32 ppt. Hatch rates were also examined for the April and May

batches. Subsamples of approximately 50 eggs were transferred volumetrically into 1 L beakers containing different concentrations of seawater. Beakers were maintained at 28 ºC in a controlled-temperature room. Three replicate beakers were used for each salinity tested. Two hours after transfer into beakers the eggs on the surface were counted. Approximately 18 hours after fertilisation the contents of each beaker was strained through a 200 µm mesh sieve. The number of hatched (larvae) and unhatched eggs was then counted under a dissecting microscope.

In February 2014 the buoyancy of eggs was tested over a wider range of salinities. The salinities tested ranged from 28-36 ppt and were tested at 2 ppt intervals. Fertilised eggs were stocked volumetrically at approximately 50 eggs per beaker. One hour after stocking beakers, the eggs floating on the surface of each beaker were counted. These were classed as buoyant eggs.

Exact counts of unhatched eggs and hatched larvae were made at the end of the experiment, post hatch, by collecting eggs in a 125 µm sieve, and counting all unhatched eggs and hatched larvae. The number of larvae and number of buoyant eggs counted were converted to percentages of the total numbers of eggs stocked. Three replicates were used for each salinity level.

Evaluating egg quality

Morphometrics

In the 2012-2013 spawning season, a subset of eggs from the first spawn of each induction event from both the standard and invertebrate diet groups was removed from the egg collection basket and taken for analysis. Morphological analysis was conducted within two hours post spawning to ensure eggs were in the 4- to 32-cell development window. This ensured morphometric measurements were standardised. Measurements were conducted using a Nikon SMZ-10A stereo microscope and measured using a 0.01 mm objective micrometer. Digital photos were taken of twenty individual eggs to analyse maximum and minimum diameters of the egg, yolk and lipid vesicle (Figure 1.3) using QCapture pro 5.1® graphics programme.

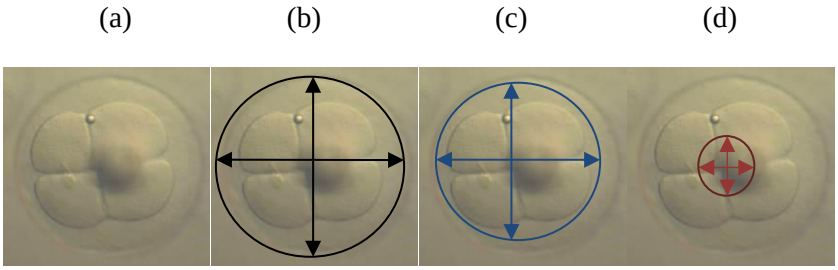

Figure 1.3: Analysis of morphological features using QCapture pro 5.1®

(a) egg showing yolk and lipid vesicle, (b) measurement of egg diameter, (c) measurement of yolk diameter, (d) measurement of lipid vesicle.

The maximal and minimal axis of each ellipse was calculated using the QCapture pro® measurement tool. These measurements were used for the calculation of several morphological indices (Table 1.3). The shape coefficient is a measure of the ratio of maximal to minimal diameters to each other and provides a measure of how spherical the morphological feature is. A shape coefficient of zero indicates a spherical shape, while increasing deviations indicate an ellipsoid (Giménez *et al.* 2006).

After the 2012-2013 spawning season, eggs were viewed with a Nikon Eclipse Ti inverted compound microscope using the 4x objective, set to PHL with phase contrast. An image was captured by a Nikon digital camera mounted on the microscope and displayed on a computer screen. Egg diameters were measured in microns using NIS Elements software. The measured digital images were saved by NIS Elements as .jpg files and the values obtained were used to determine a mean egg diameter for

individual spawns. Egg sizes were generally only measured during experimental spawning runs, but they were opportunistically measured on some production runs also.

Table 1.3: Calculated morphological indices 2012-2013 spawning season

Calculated Morphological Indices	Notation and Formula
Mean Diameter Egg	MeanDE = (MaxDE + MinDE)/2
Mean Diameter Yolk	MeanDY = (MaxDY + MinDY)/2
Mean Diameter Lipid Vesicle	MeanDL = (MaxDL + MinDL)/2
Shape Coefficient of egg	SCE = ((MaxDE - MinDE)/(MaxDE + MinDE)) x100
Shape Coefficient of yolk	SCY = ((MaxDY - MinDY)/(MaxDY + MinDY)) x100
Shape Coefficient of lipid vesicle (oil droplet)	SCO = ((MaxDL - MinDL)/(MaxDL + MinDL)) x100

DE (Diameter egg), DY (Diameter yolk), DL (Diameter lipid vesicle).

Biochemical composition

The eggs analysed for biochemical composition in this study were all preserved within the 2-32 cell cleavage stage (within 30 minutes of spawning at 28 °C) and were collected in the 2012-2013 spawning season. The egg collection basket was regularly checked for evidence of spawned eggs and once observed the eggs were removed rinsed with 0.9% ammonium formate to prevent salt crystallisation then placed in an Eppendorf container and immediately stored at -80 °C until analysis. Five replicate groups of eggs were collected per assay. The amount of eggs used per replicate for each assay was as follows: Total protein (20µg dry wt), total lipid (100µg dry wt) total fatty acid (100µg dry wt) total amino acid (100µg dry wt).

Laboratory analysis on total protein, total lipid, free amino acid and fatty acid composition was conducted at Commonwealth Scientific and Industrial Research Organisation (CSIRO) Marine and Atmospheric Research Laboratory division (Brisbane, Australia).

Dry weight and moisture content

Jungle perch eggs were weighed placed into pre-baked 550 °C and pre-weighted crucibles. Eggs were weighed to the nearest 0.1 mg and placed in the oven at 105 °C overnight. The material was removed from the oven and placed into a desiccator at 2 Torr (unit of pressure). The remaining material was left to equilibrate in the desiccator for one hour. Once the vacuum was released the crucibles were immediately re-weighed. The material was put in a muffle furnace at 200 °C for two hours and then at 550 °C overnight. The remaining material was allowed to cool inside the furnace to 100 °C before being removed from the furnace and placed in a desiccator at a vacuum of 2 Torr.

Total Lipid Extraction

Analysis for the isolation and purification of total lipids from animal tissue involves extraction of lipids following homogenisation of the tissue samples. The second step involves isolating tissues and non-tissue substances through a washing procedure (Folch *et al.* 1957). Tissue samples were weighed and placed into a 10 mL glass vial, containing 0.5 mL distilled water and 6 mL of 2:1 methanol:chloroform. The material was homogenised then placed into a 20 mL syringe with glass microfiber filter paper in the bottom and gravity filtered into a 15 mL culture tube. The material was washed along with the vial and the Ultra-turrax® shaft by addition of 6 mL of 2:1 methanol:chloroform to the vial and re-homogenisation for 20 seconds.

A syringe plunger was used to eject the residual solvents from the syringe, then 3 mL of 1% NaCl was added and mixed thoroughly by inversion and centrifuge for 5 min at 2500 rpm. A quarter of the

volume of 1:1 MeOH: 1% NaCl was added to each material and then mixed thoroughly by inversion and centrifuge for 5 min at 2500 rpm. The material was then filtered through a Na_2SO_4 filter (a 20 mL syringe, with 3 mL Na_2SO_4) into a 10 mL volume flask. A small volume of chloroform was added to the mixture to make a 10 mL solution. 1.8 mL of material was placed in a pre-weighed vial with the contents dried under a stream of nitrogen for 15 min before being placed at a vacuum of 1 Torr for 1 h.

Fatty Acid extraction

Analysis of fatty acids was conducted using the FAME protocol, a one-step extraction and derivatisation procedure for the analysis of fatty acids from animal tissue. The protocol relies on an acid catalysed transesterification reaction (Coutteau & Sorgeloos 1995). An aliquot containing 3 mg of total lipid was transferred to a 15 mL Pyrex screw cap test tube and dried under nitrogen. 1 mL of MeOH: Toluene (3:2) and 1 mL of Acetyl chloride: Methanol (1:20) was added to the test tube. The test tube was placed in a heating block at 100 °C for 1 h and when cooled was added as an internal standard and washed into the tube with 1.5 mL hexane and 1 mL water. The test tubes were centrifuged at 1500 rpm for 2 min before pipetting off the upper layer and filtering it through Na_2SO_4 into a 4.5 mL high performance liquid chromatography vial. The hexane extraction was repeated twice more without additional centrifuging. The upper layer was mixed and pipetted into a 1.5 mL vial for gas chromatography analysis. Gas chromatography was analysed using the Agilent method (Frank *et al.* 2002).

Total Protein extraction

Nitrogen was analysed using dynamic flash combustion (Sweeney 1989). The material was inserted into a tin capsule, sealed and placed into a combustion reactor at 900 °C. A small volume of pure oxygen was added to the system to combust all organic and inorganic material, converting the material into elemental gasses. A separation column and thermal conductivity detector were then used to determine element concentrations. Total nitrogen was then converted to protein by a multiplier of 6.25. This process was conducted three times; if the variance of these three values was less than 10% the mean was taken and reported. If the variance was greater than 10% the sample was reanalysed.

Embryological development

Embryological development was observed and recorded in the 2013-14 spawning season. During a spawn the egg collector was checked every 15 min for spawned eggs. Once observed, eggs were removed gently, placed in a 20 L container, mixed gently then allowed to settle. Negatively buoyant eggs were discarded prior to analysis and excluded from further sampling.

Approximately 5000 eggs were removed from the spawned batch, transferred to 10 L aquaria and maintained at 28 $^{\circ}$C ± 0.5°C for observations of embryonic development. Eggs were observed for the period immediately following spawning to hatch using a Nikon Eclipse Ti inverted microscope. Embryonic developmental stages were identified according to Blaxter (1969) and Friogeirsson (1978), and were captured digitally using a DS-U3 Nikon colour video camera (Nikon Imaging Software version 4.13). Cell divisions were measured using 10 x Differential Interference Contrast (DIC) microscopy. Cell division was considered to have reached a specific developmental stage when more than 50% of a 5 mL egg sample had reached that stage.

Assessing effectiveness of iodine treatment of eggs

It was thought that vertical transmission of disease could be an issue, compromising survival of early larvae. Therefore iodine treatment of eggs was investigated to see if this could be a viable procedure to reduce disease risk in early-stage larvae. The iodine experiments were spread over two spawnings. The first evaluated a greater range of doses of iodine, the second narrowed the focus to a range of lower doses.

- Approximately 20 000 embryos were removed from a hatching tank and placed in a bucket with 10 L seawater (approx. 2000 larvae.L^{-1}).
- The number of embryos was estimated from 10 mL sub-samples to enable volumetric allocations.
- A volume containing approximately 2000 embryos was transferred to a 20 µm screen in the base of a bucket of UV-treated 1 µm-filtered seawater.
- The bucket was drained slowly (2 min) to strand eggs on the screen.
- Eggs were transferred from the screen to a bucket with selected iodine concentration in the first spawning at iodine concentrations of 0 mg.L^{-1}, 25 mg.L^{-1}, 50 mg.L^{-1} and 100 mg.L^{-1}, and at concentrations of 0 mg.L^{-1}, 15 mg.L^{-1}, 20 mg.L^{-1} and 25 mg.L^{-1} in the second spawning. There was also an untreated control group that was incubated at a concentration of 0 mg.L^{-1} iodine without screen manipulation in each spawning.
- The embryos were gently mixed for 5 min by slowly rotating the screen every 30 sec.
- After the exposure period the bucket was drained slowly (2 min) to strand eggs on the screen (draining started after 3 min exposure).
- The drained screen was transferred to a bucket of clean UV-treated seawater and swirled for 1 min.
- The bucket was drained slowly (2 min) to strand eggs on the screen.
- The eggs were washed into an incubation bowl containing 1 L UV-treated seawater and topped up to 2 L (approx. 2000 eggs in 2 L).
- 3 hours after hatching commenced, 100 mL samples were taken from well-mixed bowls and the number of hatched larvae were counted.

There were three replicate samples per spawning for each iodine dose, except in the first spawning, when only one replicate was used for the 25 mg.L^{-1} dose. Only one replicate per spawn was used for the no-screen manipulation control in both spawnings.

Statistical analyses

Buoyancy and hatch rates at different salinities

Data on egg buoyancy at different salinities were analysed using a generalised linear model (GLM) of binomial proportions with a logit link function (using GenStat® 15th edition software package, VSN International Limited), followed by a post hoc pairwise least significant differences (LSD) test. For salinity experiments between 28 and 32 ppt which involved several batches, the effect of batch was also analysed in the GLM.

A generalised linear model of binomial proportions with logit link function was also run for hatch rate against salinity. This was also followed by a post hoc LSD test.

Egg morphometric data

Statistical analysis on egg morphometric data from the 2012/13 spawning season (November to May) was conducted using GenStat®. The interactions across months, between diets (Standard vs domesticated), broodstock source (Mackay-Townsville vs Fraser) and between 'wild' and domesticated jungle perch were analysed using a four-way unbalanced ANOVA with two-tailed test of significance. 'Wild' fish were those that had been in captivity for less than six months. Domesticated fish had been in captivity for periods greater than 12 months, and in most cases for greater than three years. For the experimental spawns, linear regression analysis was used to test for any relationships between the following variables: mean egg diameter and hatching rate; mean egg diameter and total eggs spawned; egg, yolk and lipid vesicle shape coefficient with hatching and fertilisation rate; egg diameter and yolk diameter; and egg diameter and lipid vesicle diameter across diet groups. Using additional data collected from production runs, further linear regression analyses were run to compare egg diameter with number of eggs spawned, egg diameter with broodstock size and egg diameter and egg shape coefficient with fertilisation rates. Diet was not a component of these latter analyses. A set

of spawns that took place during a low-salinity event in early 2013 was excluded from the analyses because reduced salinity can impact on fertilisation and hatch rates.

Egg biochemistry

Statistical analyses on biochemical composition were conducted using GenStat®. Biochemical composition was calculated based on the quantity of components per egg sample and based on egg sample volume. Tanks were taken as independent experimental units with the data subjected to a main effects unbalanced analysis of variance. The fitted terms were time, source location of broodstock and diet treatment. Regression analysis was used to determine relationships in total protein, total lipid, total free amino acid and total fatty acid content between diets over the spawning season. Linear regression was used to test for any relationships between the biochemical components and hatch rates or fertilisation rates. ANOVA was used to determine if there were any significant differences between treatments for the different biochemical components.

Iodine treatment of eggs

The iodine treatment data were analysed using a GLM of binomial proportions, with a logit link function (using GenStat®), followed by a post hoc pairwise LSD test of back-transformed values.

Results

Latency times and fertilisation rates

Latency times from induction to spawning for fish held at 28 ºC in seawater were close to normally distributed (Figure 1.4). The mean latency period was 55 h 21 min and the median latency period was 54 h 6 min.

Fertilisation rates were not normally distributed (Figure 1.5), with a large number of samples have fertilisation rates above 50% and a peak number of samples having fertilisation rates less than 5%. The mean fertilisation rate was 56.6% and the median fertilisation rate 65% (meaning that more than half the spawns had fertilisation rates greater than 65%).

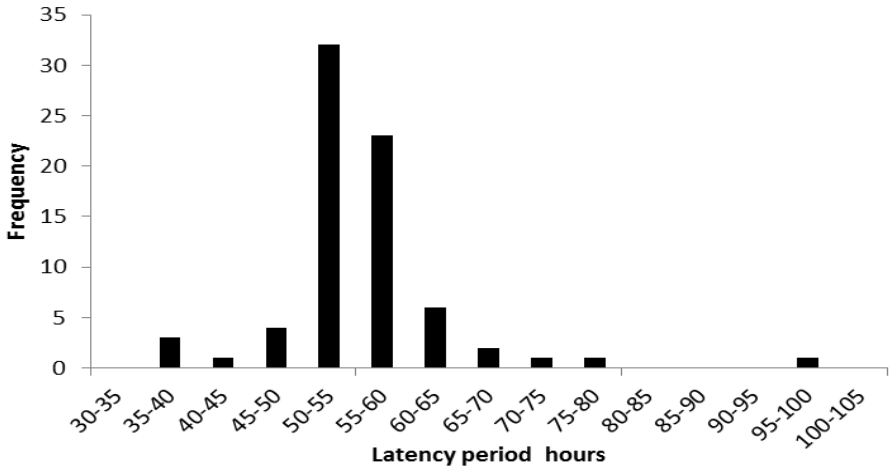

Figure 1.4: Frequency histogram of spawning latency periods for jungle perch spawned at 28 ºC in seawater.

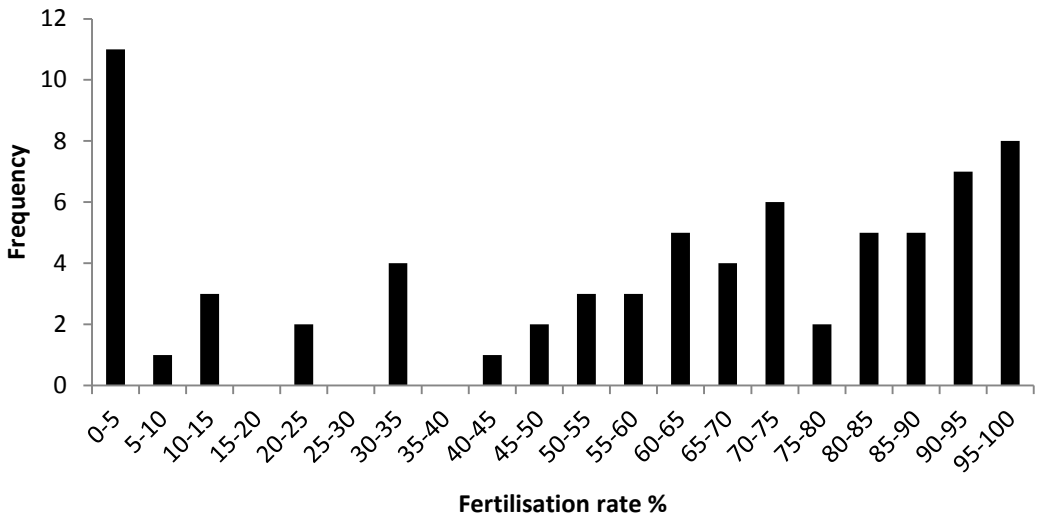

Figure 1.5: Frequency histogram of fertilisation rates for jungle perch eggs spawned at 28 ºC in seawater.

Effects of salinity on egg buoyancy and hatch rate

For the egg buoyancy experiments at salinities between 28 and 32 ppt the results are summarised in Tables 1.4 and 1.5 and in Figures 1.6 and 1.7.

Table 1.4: Significance of factors batch, salinity and batch.salinity on the per cent of buoyant eggs.

Source of variation	Degrees of freedom	ss	ms	v.r.	F pr.
Batch	2	635.9	317.95	13.06	<0.001
Salinity	3	4632.5	1544.16	63.43	<0.001
Batch.Salinity	6	1628.3	271.38	11.15	<0.001

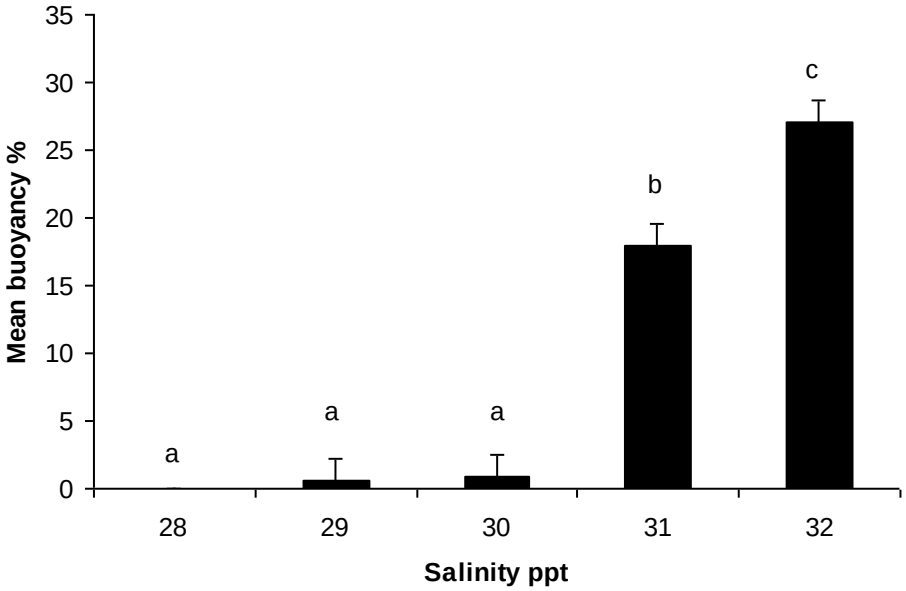

Figure 1.6: Mean buoyancy of jungle perch eggs at different salinities for the batches combined.

Salinity categories with letters in common are not significantly different. Categories not sharing the same letter are significantly different at the 5% probability level.

Table 1.5: Significance of factors batch, salinity and batch.salinity on the per cent of hatched eggs.

Source of variation	Degrees of freedom	ss	ms	v.r.	F pr.
Batch	1	12214.15	12214.15	324.5	<0.001
Salinity	4	650.45	162.61	4.32	<0.011
Batch.Salinity	4	606.60	151.65	4.03	<0.015

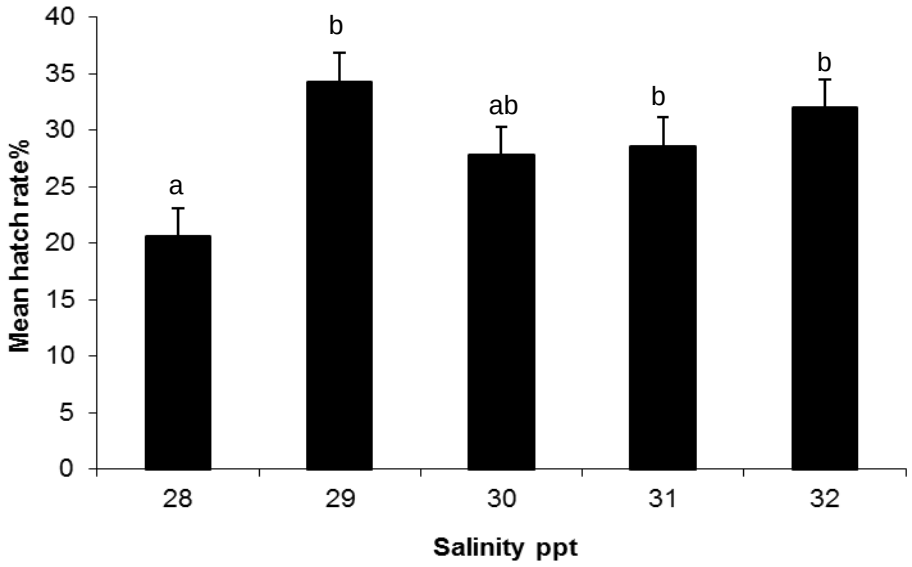

Figure 1.7: Mean hatch rate (%) of jungle perch eggs at different salinities for the batches combined. Salinity categories with letters in common are not significantly different. Categories not sharing the same letter are significantly different at the 5% probability level.

Egg buoyancy was poor below 31 ppt and was significantly higher at 32 ppt than at 31 ppt. Hatch rates were poorer at 28 ppt than at higher salinities. There were significant differences in buoyancy rates (particularly above 31 ppt) and hatch rates between batches of eggs. This may have been due to differences in egg quality between batches.

In the second set of salinity tests for salinities between 28 ppt and 36 ppt, all eggs at 28 ppt and 30 ppt were observed on the bottom of the beaker. Only eggs seen on the surface were counted as buoyant. However, in salinities of 32 ppt and above, neutrally buoyant eggs were observed in the water columns of the test beakers. These were not counted in the buoyancy test due to difficulties in accurately counting them. However, no neutrally buoyant eggs were observed at 28 ppt or 30 ppt, with all eggs in those beakers being on the bottom. Hatch rates in the 28 ppt and 30 ppt beakers were about half or less than half those at 32 ppt, 34 ppt and 36 ppt (Figure 1.8). The GLM of binomial proportions for egg buoyancy was significant, followed by a post hoc pairwise LSD test. The relationship was significant (Table 1.6). Post hoc LSD analyses show that eggs were significantly more buoyant at salinities of 32 ppt and above than at lower salinities, with no buoyant eggs detected at 30ppt or less. Buoyancy was significantly higher at 36 ppt than at all other salinities tested (Table 1.7). There was no significant difference in buoyancy between salinities in the range 32 ppt to 34 ppt (Table 1.7).

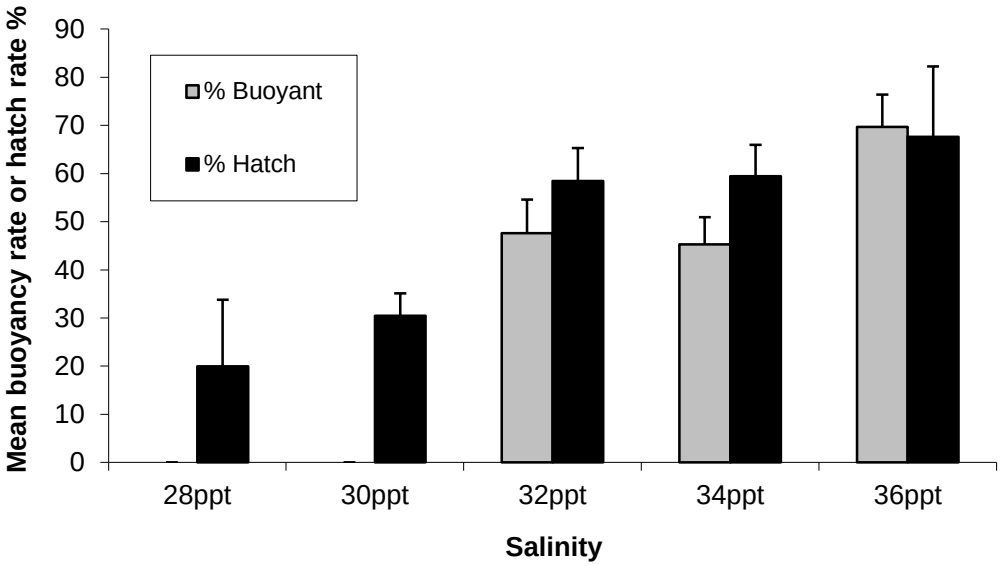

Figure 1.8: Effect of salinity on egg buoyancy and larval hatch rates.

Error bars show on standard error of the mean (n=3) Statistical differences for buoyancy and hatch rates at different salinities are shown in tables 1.7 and 1.9 respectively.

Table 1.6: Summary of regression analysis of binomial proportions with logit link function for egg buoyancy at different salinities.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx. F pr.
Regression	4	345.94	86.485	63.64	<.001
Residual	10	13.59	1.359		
Total	14	359.53	25.681		

Table 1.7: Post hoc LSD test for egg buoyancy rates at different salinities. Means with the same subscript are not significantly different at the P = 0.050 level

Salinity ppt	Mean proportion of buoyant eggs
28	0.0000 a
30	0.0000 a
32	0.4686 b
34	0.4545 b
36	0.7017 c

The GLM of binomial proportions with logit link function was significant for hatch rate against salinity (Table 1.8). The post hoc LSD test showed that hatch rates were significantly lower at 28 ppt compared to salinities of 32 ppt or higher (Table 1.9). Hatch rates were significantly higher at 34 ppt and 36 ppt compared to salinities of 30ppt or lower (Table 1.9).

Table 1.8: Summary of regression analysis of binomial proportions with logit link function for egg hatching rates at different salinities.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx. F pr.
Regression	4	109.08	27.270	4.81	0.020
Residual	10	56.74	5.674		
Total	14	165.83	11.845		

Table 1.9: Post hoc LSD test for egg hatching rates at different salinities. Means with the same subscript are not significantly different at the P=0.050 level.

Salinity ppt	Mean proportion of hatched eggs
28	0.2000 a
30	0.3158 ab
32	0.5771 bc
34	0.5960 c
36	0.7017 c

Egg morphometry with reference to broodstock characteristics and spawning

Results for the key factors of interest, broodstock source group, and broodstock diet group and broodstock type are presented in Table 1.10.

The key egg quality parameters were not significantly different between broodstock source groups (Fraser vs. Mackay-Townsville). However, male size was significantly different between the two groups. This is probably not biologically significant, merely a reflection of more young males in the Mackay-Townsville group.

Only one egg quality parameter was significantly different between the invertebrate diet group and the standard diet group. The egg shape coefficient indicated the standard diet group tended to have more consistently spherical eggs than the invertebrate diet group ($p = 0.018$). Both male and female broodstock size were significantly different statistically between the two groups. Again this is probably not biologically significant.

There was a significant difference between egg quality parameters of 'wild' and domesticated broodstock. Apart from hatching rates, all other parameters were significantly different between the two groups. Wild fish had significantly larger eggs, larger yolk and oil volumes, and more spherical eggs (Table1.10). However the 'wild' sample was small compared to the standard and invertebrate diet groups. The factor month is not shown in Table 1.10, but month had no significant effect on any of the key variables.

Table 1.10: Hatching rate (%), egg, yolk and lipid vesicle diameter (µm), and shape coefficient for egg and lipid vesicle across source locations, diet treatments and wild vs domesticated *K. rupestris* in the 2012/13 spawning season. P values are as derived from ANOVA.

	Range	Mean ± SE	Range	Mean ± SE	P Value
Source group		Fraser		Townsville	
Hatching (%)	46 - 67	62 ± 15	0 - 99	69 ± 14	P4,9 = 0.753
Egg diameter (µm)	620 - 676	650 ± 10.5	650 - 677	666 ± 9.6	P4,9 = 0.956
Egg shape coefficient	1.15 - 1.87	1.44 ± 0.08	1.44 - 1.68	1.56 ± 0.74	P4,9 = 0.250
Lipid vesicle diameter (µm)	170 - 182	181 ± 2.5	167 - 183	174 ± 2.3	P4,9 = 0.108
Lipid shape coefficient	0.90 - 3.65	1.8 ± 0.36	0.96 - 4.26	2.27 ± 0.33	P4,9 = 0.392
Yolk diameter (µm)	598 ± 648	625 ± 9.6	618 ± 649	638 ± 8.7	P4,9 = 0.353
Diet types		Invertebrate		Standard	
Hatching (%)	45 - 62	49 ± 20	0 - 99	75 ± 9	P4,9 = 0.170
Egg diameter (µm)	620 - 677	646 ± 9.5	622 - 677	670 ± 8.7	P4,9 = 0.132
Egg shape coefficient	1.54 - 1.85	1.73 ± 0.73	1.15 - 1.68	1.35 ± 0.06	P4,9 = 0.018*
Lipid vesicle diameter (µm)	170 - 180	176 ± 2.2		178 ± 2.0	P4,9 = 0.564
Lipid vesicle shape coefficient	1.36 - 4.05	2.67 ± 0.33	0.91 - 2.03	1.54 ± 0.30	P4,9 = 0.063
Yolk diameter (µm)	598 ± 649	618 ± 8.7	594 ± 648	644 ± 7.9	P4,9 = 0.085
Type		Wild		Domesticated	
Hatching (%)	97	97 ± 0	0 - 99	62 ± 11	P4,9 = 0.259
Egg diameter (µm)	681 - 752	723 ± 24.5	536 - 722	652 ± 7.8	P4,9 = 0.052
Egg shape coefficient	0.00 - 1.96	0.93 ± 0.188	0.00 - 5.70	1.58 ± 0.06	P4,9 = 0.030*
Lipid vesicle diameter (µm)	184 - 205	198 ± 5.75	167 - 183	175 ± 1.8	P4,9 = 0.019*
Lipid vesicle shape coefficient	0.00 - 1.90	0.78 ± 0.84	0.00 - 20.2	2.31 ± 0.27	P4,9 = 0.032*
Yolk diameter (µm)	653 ± 745	700 ± 22.2	598 ± 649	625 ±7.02	P4,9 = 0.032*

Some of the morphometric measures of egg quality, such as egg diameter, were correlated with hatching success ($R^2 = 0.74$, $R = 0.86$, $p < 0.01$, Figure 1.9). The number of eggs spawned was negatively correlated with egg size across all spawning seasons ($R^2 = 0.16$, $R = -0.40$, $p < 0.01$, Figure 1.10). Yolk diameter was significantly correlated with egg diameter (Figures 1.11 and 1.12). The relationship was similar for eggs produced by broodstock on the standard diet ($R^2 = 0.81$, $R = 0.90$, $p < 0.001$), and the invertebrate diet ($R^2 = 0.88$, $R = 0.94$, $p < 0.001$). There was no significant difference between the two diet groups for this morphometric relationship. In the 2012/13 spawning season fertilisation rate was also significantly correlated with egg diameter ($R^2 = 0.54$, $R = 0.73$, $p<0.01$). Fertilisation rate was also strongly correlated with hatching rate ($R^2= 0.87$, $R= 0.93$, $p<0.01$). However, the combined spawn data from the 2012/13, 2013/14 and 2014/15 spawning seasons showed no significant relationship between egg diameter and fertilisation rate ($R^2 = 0.004$, $R = 0.06$, $P > 0.05$). In the 2012/13 season spawning females were stocked three to a tank with nine males and egg quality data were collected for the first spawn in each tank. In subsequent seasons females were mostly stocked one to a tank with three males. If male contribution to spawning was a problem, the first female to spawn in a tank with nine males, would have a greater chance of spawning with a suitable male than a female in a tank with just three males. Therefore it is possible that in the latter part of the project, females with large eggs of good quality may have had an increased probability of having spawns compromised by poor male performance.

There were no significant correlations between lipid droplet size and hatch rate or fertilisation rate, or between lipid shape coefficient and egg shape coefficient. Egg shape coefficient and lipid shape coefficient also showed no significant correlation between hatch rate and fertilisation rate.

Lipid droplet size was positively correlated with both egg diameter for both broodstock diet groups (Invertebrate diet $R^2 = 0.13$, $R = 0.37$, $p < 0.001$; Standard diet $R^2 = 0.13$, $R = 0.36$, $p < 0.001$). Lipid droplet size was also positively correlated with yolk diameter for each of the diet groups (Invertebrate diet $R^2 = 0.13$, $R = 0.36$, $p < 0.001$; Standard diet $R^2 = 0.12$, $R = 0.34$ $p < 0.001$).

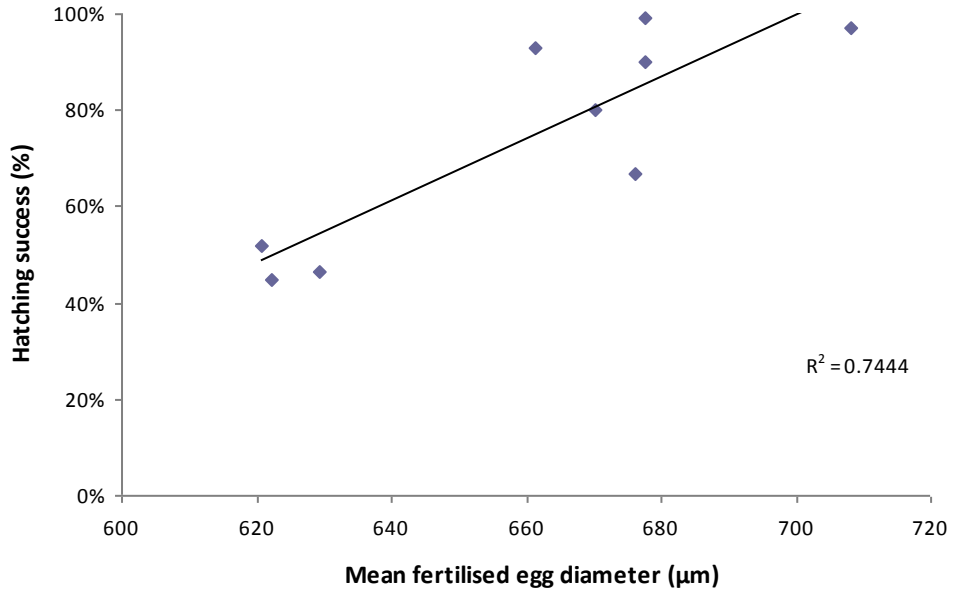

Figure 1.9: The relationship between mean egg diameter of a spawn and hatching success 2012/13 season.

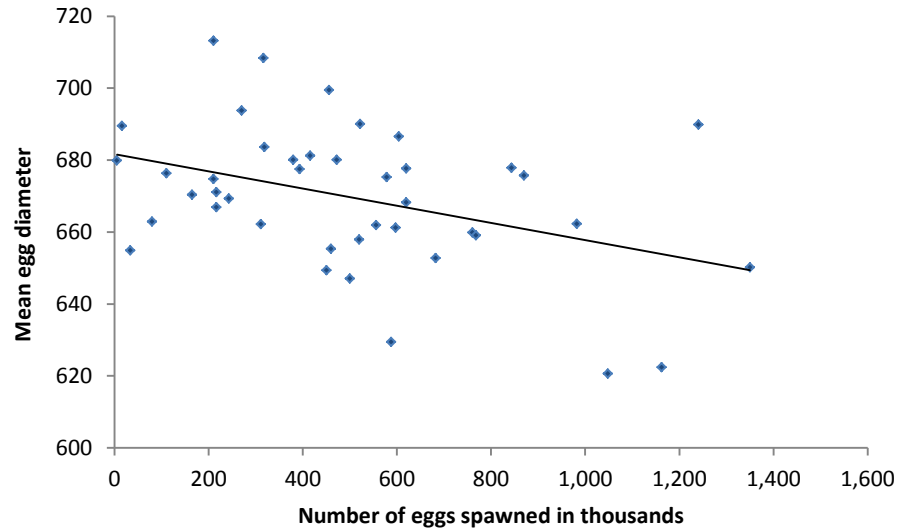

Figure 1.10: The relationship between egg size and number of eggs spawned 2012-2015.

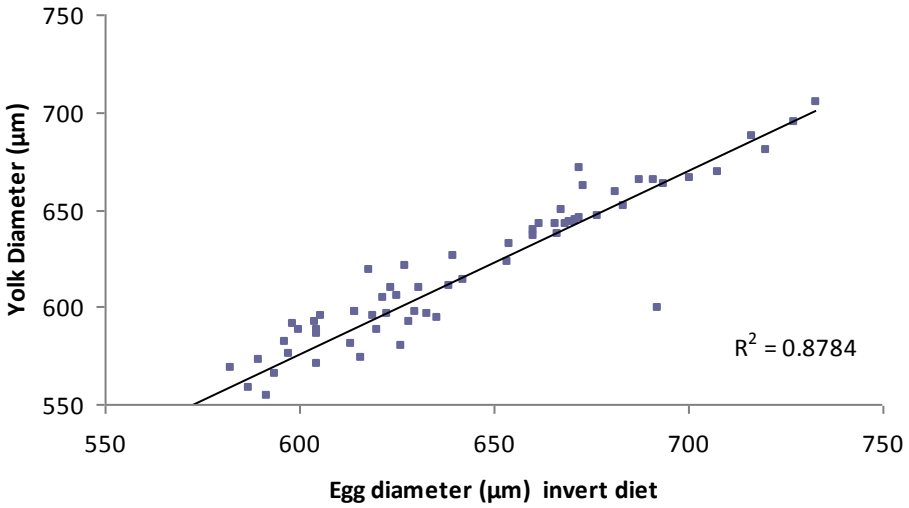

Figure 1.11: The relationship between egg diameter and yolk diameter of eggs from the invertebrate diet group 2012/13 season.

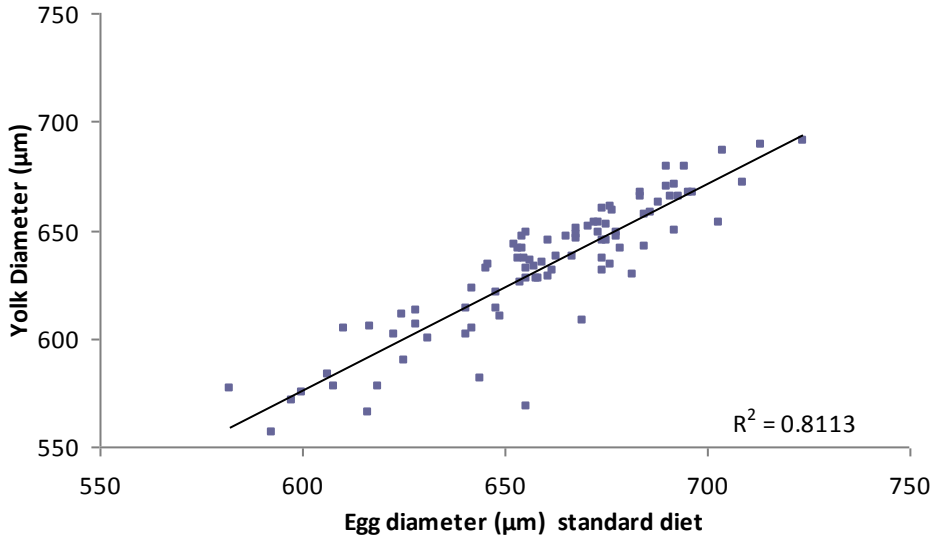

Figure 1.12: The relationship between egg diameter and yolk diameter of eggs from the standard diet group 2012/13.

Biochemical composition of eggs

Jungle perch broodstock were induced to spawn from November 2012 through to May 2013. A total of approximately 18 million eggs were released from nine spawning events comprising a total of 30 females during this study. Eggs that were collected for analysis were from the first spawns each month of broodstock on the Invertebrate and Standard diets.

Invertebrate Diet
Fertilisation rates ranged from 0 to 98% (mean = 53 ± 20%) while the hatching rates ranged from 0 to 90% (mean = 49% ± 20%) (Table 1.11).

Marine/Aquatic (Standard) Diet
Fertilisation rate was significantly higher on the standard diet ranging from 77 to 99% (mean = 83% ± 3%). Although the mean hatching rate was higher on the standard diet (Table 1.11) (mean = 75% ± 9%), the difference was not significant; note that the range of hatching rates was far wider (0 to 90%).

Table 1.11: Hatching rate, fertilisation rate and total eggs across diets over the 2012/13 spawning season (range and mean ± SEM).

	Invertebrate Diet		Standard Diet		
	Range	Mean ± SE	Range	Mean ± SE	P value
Hatching rate (%)	45 - 62	49 ± 20	0 - 99	75 ± 9	$P_{5,9} = 0.170$
Fertilisation rate (%)	0 - 98	53 ± 20	77 - 90	83 ± 3	$P_{5,9} = 0.042$

Correlations of biochemical components

Total lipid content and total protein content were significantly positively correlated ($r = 0.477$, $P < 0.05$), indicating that an increase of lipids in the egg was associated with an increase in protein. There was a significant negative relationship between egg size and total protein content ($r = -0.45$, $P < 0.05$), indicating decreased egg size is associated with a higher percentage of total protein in the egg. Total lipid and free amino acid did not show any significant relationships to other components. There was no significant temporal trend in protein, lipid or dry matter over the spawning season (Figure 1.13).

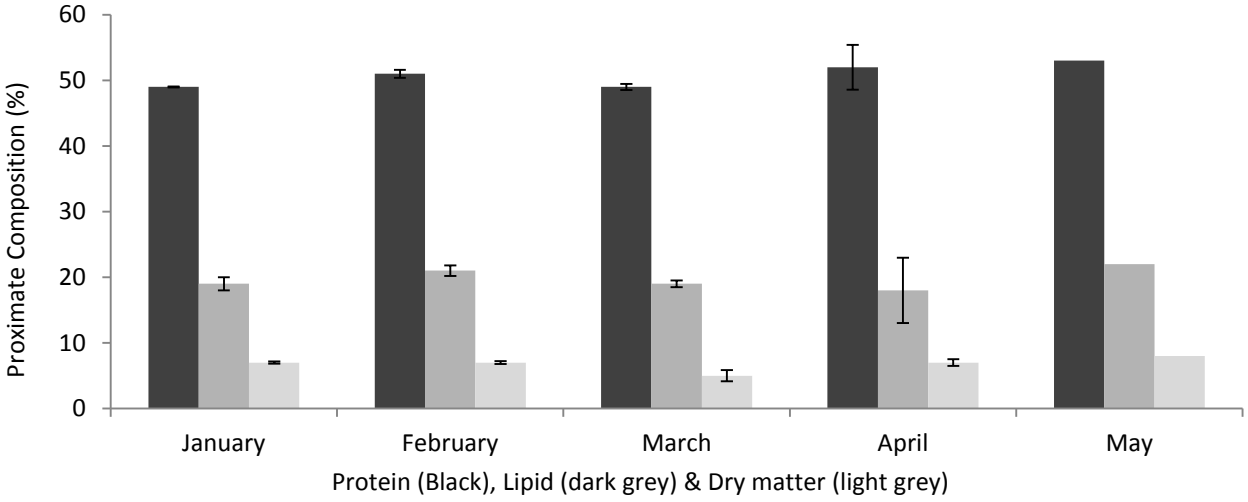

Figure 1.13: Temporal variations in protein, lipid and dry matter over 2012/13 spawning season across diet treatments (mean ± SEM).

Allocation of biochemical components

Invertebrate diet

Total protein ranged from 48.5 - 54.0% of the total biochemical content (mean of 50.7% ± 1.5%). Total lipid ranged from 19.1 to 23.1% (mean of 21.2% ± 0.9%). The pool of free amino acids fluctuated from 26.7 to 108.6 mg/g (mean of 76.04 mg/g ± 21.63 mg/g) (Table 1.12). The relative

composition of the eggs over the season fluctuated considerably with a peak in amino acid content in February followed by a steep decline in March. The rest of the season showed higher values.

Marine/Aquatic (Standard) diet

Total protein ranged from 48.10 - 51.83% of the total biochemical content (mean of 49.79% ± 0.67%). Total lipid ranged from 13.12 to 21.25% (mean of 18.15% ± 1.40%). The pool of free amino acids ranged from 21.41 to 109.98 mg/g (mean of 68.52 mg/g ± 17.62 mg/g) (Table 1.12). The relative composition of the eggs over the season fluctuated considerably with a peak in amino acid content in April, with March showing the lowest amino acid content.

Table1.12: Proximate composition of jungle perch eggs across broodstock diet treatments over the 2012/13 spawning season range and mean ± SEM.

	Invertebrate Diet		Standard Diet		
	Range	Mean ± SE	Range	Mean ± SE	P value
Dry Matter (%)	4.512 - 7.916	6.684 ± 0.768	6.230 - 7.265	6.969 ± 0.196	$P_{5,9} = 0.915$
Total Protein (%)	48.449 - 54.969	50.725 ± 1.481	48.097 - 51.826	49.791 ± 0.667	$P_{5,9} = 0.386$
Total Lipids (%)	19.118 - 23.087	21.168 ± 0.927	13.129 - 21.250	18.152 ± 1.397	$P_{5,9} = 0.060$
Free amino acids (mg/g)	26.952 - 108.614	76.039 ± 21.631	21.405 - 109.976	68.515 ± 17.624	$P_{5,9} = 0.964$

Variation in fatty acid composition across diet treatments

Most of the fatty acid groups were significantly different between eggs from the two diet treatments, except for Arachidonic acid (ARA), myristic acid 14:0 and stearic acid 18:0 (Table 1.13). The saturated fatty acid, palmitic acid (16:0) was the most abundant fatty acid in the eggs of jungle perch fed both broodstock diets. However, Palmitic acid was significantly higher in eggs from the invertebrate diet group than in eggs from the standard diet group (Table 1.13; Figure 1.14). The most abundant unsaturated fatty acids in the eggs from both diet groups included the monounsaturated oleic acid (18:1n - 9C), the polyunsaturated linoleic acid (18:2n - 6) and docosahexaenoic acid (22:6n -3) DHA (Figure 1.14; Table 1.13).

The invertebrate diet group had eggs with significantly lower n-HUFA fatty acid proportion, and higher levels of linoleic (18:2n -6) and oleic acid (18:1n - 9C) than standard diet group eggs (Table 1.13). In comparison, significantly higher quantities of Docosahexaenoic acid (22:6[n-3]) DHA, eicosapentaenoic acid (20:5n - 3) (EPA), docosapentaenoic acid (22:5n - 3) and n-3 HUFA were observed in eggs from the standard diet group compared to the invertebrate diet group (Table 1.13; Figure 1.14).

Table 1.13: Fatty acid percentage composition in jungle perch eggs across broodstock diet treatments (mean ± SEM). For the notation used in the fatty acid column, the number to the left of the colon is the number of carbon atoms in the compound, the number immediately to the right of the colon is the number of double bonds, and the number after the hyphen indicates the position of the first double bond from the methyl end.

Fatty Acid	Invertebrate Diet	Standard Diet	P = Value
14:0	2.99 ± 0.60	4.14 ± 0.20	$P_{2,9} = 0.06$
16:0	28.79 ± 1.13	23.90 ± 0.51	$P_{2,9} = < 0.01$*
18:0	6.54 ± 0.41	5.98 ± 0.14	$P_{2,9} = 0.22$
16:1 (n - 7)	4.28 ± 0.73	6.70 ± 0.37	$P_{2,9} = 0.03$*
18:1(trans) (n - 9)	0.31 ± 0.31	2.56 ± 0.15	$P_{2,9} = < 0.01$*
18:1(cis) (n - 9)	25.17 ± 1.11	20.75 ± 0.88	$P_{2,9} = < 0.01$*
18:2 (n - 6)	10.59 ± 0.72	6.96 ± 0.59	$P_{2,9} = < 0.01$*
20:4(n - 6) (ARA)	1.59 ± 0.57	2.40 ± 0.31	$P_{2,9} = 0.21$
20:5(n - 3) (EPA)	1.63 ± 0.68	4.63 ± 0.39	$P_{2,9} = < 0.01$*
22:5(n - 3)	2.18 ± 0.77	5.17 ± 0.39	$P_{2,9} = < 0.01$*
22:6(n - 3) (DHA)	11.66 ± 1.45	16.81 ± 0.91	$P_{2,9} = < 0.01$*
n - 3 HUFA	13.2878 ± 2.1293	21.4354 ± 1.1824	$P_{2,9} = < 0.01$*

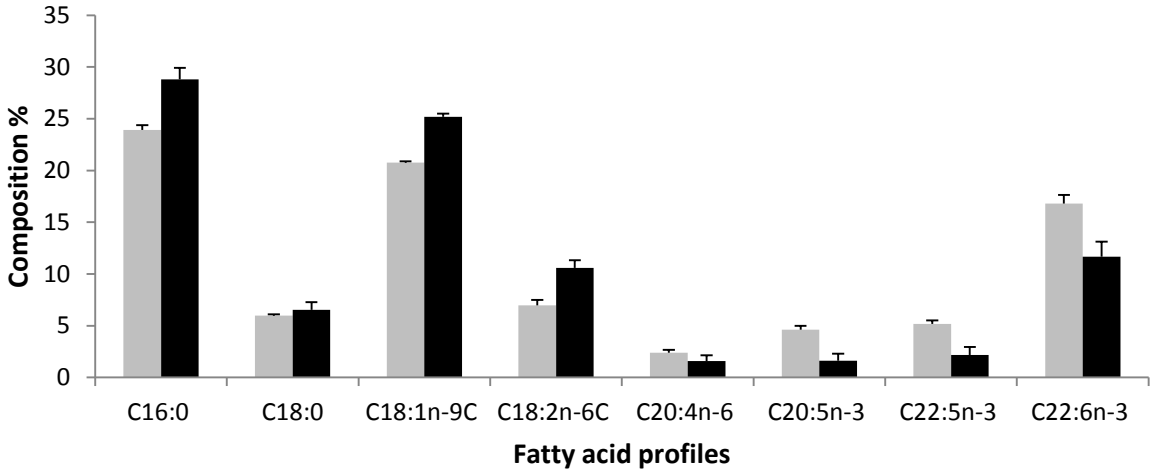

Figure 1.14: Fatty acid composition of jungle perch eggs across diet treatments for 2012/2013 spawning season mean ± SEM. Grey = standard diet (mainly aquatic components) and Black = invertebrate diet (high component of terrestrial invertebrates)

The correlation between the various dietary components and hatching success is shown in Table 1.14. Only two components were significantly correlated with hatching success. Saturated fatty acids (SFA) were negatively correlated with hatching success (p=0.034) and eicosapentaenoic acid (EPA) was positively associated with hatching success (p=0.050).

Table 1.14: Correlation between hatching percentage and nutrient components of diet treatments * denotes a correlation significant at the 5% probability level

Component	Correlation Coefficient	Probability
Lipid	-0.284	0.495
Protein	-0.094	0.819
Free Amino Acid	0.370	0.366
SFA	-0.774	*0.034
MUFA	-0.554	0.163
PUFA	0.007	0.996
ArA (20:4n - 6)	-0.137	0.745
DHA (22:6n - 3)	0.371	0.196
EPA (20:5n - 3)	0.645	*0.050
EPA + DHA	0.526	0.103
Sum n - 3	0.620	0.060
Sum n - 6	-0.215	0.609

Embryological development

The chronology of embryological development is provided in Table 1.15. Times are expressed as decimal fractions of hours post fertilisation (hpf) (for incubation at 28 ºC) and as degree hours post-fertilisation. The eggs of *K. rupestris* are positively buoyant, non-adhesive, with a clear and spherical chorion and a homogeneous yolk (Figure 1.15). The diameter of fertilised eggs ranged from 620\-710 μm with a mean of 660 ± 13 μm (SEM) (N = 200). They were characterised by a single, large oil globule (170 ± 30 μm (SEM) in diameter. However, there were often multiple smaller oil globules that ranged from 10-100 μm in diameter. These eggs had a much lower hatch rate than those with a single oil globule. There was some minor granulation on the yolk mass and the previtelline space is narrow (20 μm).

Fertilisation stage

At this stage cell division has not yet begun (0 hpf), the fertilised egg is transparent, with a slight brownish hue to the yolk. Cytoplasm is located throughout the egg with slight undulations on the surface of the cytoplasm (Figure 1.15a). The small size of the egg (660 μm) together with the transparent colouration make the egg almost invisible to the human eye.

Blastodisc cleavages

Egg cleavage is meroblastic and the first cleavage (two-cell stage) was observed within 15 minutes of spawning (Table 1.15, Figure 1.15b). The blastodisc divided via meridional cleavage to form two equal-sized cells. Twenty minutes after fertilisation four blastomeres were clearly visible (Figure 1.15c). The blastodisc divided via meridional cleavage to form four equal cells. The third cleavage occurred 30 minutes post spawning with eight cells visible (Figure 1.15d). The third cleavage was equatorial in nature and resulted in eight cells located in two parallel rows of four cells. The next cleavage took place 38 minutes after spawning (Figure 1.15e). Cleavage occurred on two separate

planes, the cleavage furrow parallel to second cleavage plane and resulted in 16 cells. Fifty-two minutes after fertilisation the fifth cleavage occurred (Figure 1.15f). The blastodisc divided via meridional cleavage into 32 cells, the cells became smaller and formed into an eight by four cell configuration. The sixth and last cleavage occurred 1.12 hours after spawning. The blastodisc attained a berry-like appearance (morula) (Figure 1.15g). During these stages cells became smaller as they multiplied. The size of the blastodisc remained constant during these cell divisions. The result of this process was a morula at 1.45 hours post spawning (Figure 1.15h). Over the next two hours the morula became smaller and began to migrate towards the pole (Figure 1.15i, j, k, l). The border of the morula thickened and formed the germ ring of the blastula at 4.78 hours (Figure 1.15m).

Gastrulation

The beginning of gastrulation occurred with the formation of embryonic tissue layers 5.20 hours post spawning (Figure 1.15n). The margins of the blastodisc thickened and formed the germ ring. In one region of the germ ring the thickening continued to form the embryonic shield which determined the future axis of the embryo (6.13 hours) (Figure 1.15o). By the end of this stage the germ ring had enclosed one third of the yolk mass and the first visible signs of the embryo appeared along the median region of the embryonic shield (7.38 hours) (Figure 1.15p).

Organogenesis

The first rudimentary organs represented the end of gastrulation and the start of organogenesis. The yolk sphere was covered by the thin blastoderm leaving a small area around the vegetal pole exposed. The head and notochord can be seen developing from a dorsal view (7.56 hours) (Figure 1.15q). The first pigmented cells (melanophores) appeared in the yolk 7.9 hours post fertilisation (Figure 1.15r). The head was recognised anteriorly in the distinct embryonic body. A solid optic bud appeared (eye sockets) along with the first somites along the neural tube. Melanophores appeared throughout the egg at 8.45 hours after fertilisation (Figure 1.15s). By 9.33 hours the embryo expanded to half of the yolk sac circumference and the blastopore had closed. Somites had expanded to half of the embryonic body, and the tail had formed but was still attached to the yolk sac (Figure 1.15t).

At 9.67 hours the tail and head regions of the embryo become visible and continue developing. Melanophores had started to migrate throughout the lateral and anterior regions of the yolk and over the oil globule; somites were visible along the entire notochord (Figure 1.15u). Following this, the tail started to separate from the yolk mass and narrow as it elongated. Melanophores had fully migrated along the embryonic body by 9.91 hours (Figure 1.15v).

Pre-hatching

After 10.17 hours the tail had fully separated from the yolk and the embryo continued to grow (Figure 1w). The tail wraps around the yolk and the heart was active; embryo pigmentation continued to intensify along the body (10.67 hours) (Figure 1.15x). At 13.3 hours the embryo was three-quarters of the egg circumference with the tail moving rapidly while the rest of the embryo was attached to the yolk (Figure 1.15y).

Hatching

Embryos started to hatch approximately 15.15 hours after spawning. The embryo floats on or near the water surface with the yolk sac facing upwards (Figure 1.15i). Newly hatched larvae were approximately 1.8 ± 0.2 mm in length with unpigmented retinas surrounding a spherical lens (15.41 hours). The body is heavily pigmented with melanophores except for the region directly behind the head. The otoliths were clearly visible in the head region with primordial fins present (Figure 1.15 II).

Table 1.15: Chronology of K. rupestris egg development at 28oC in both degree hours post fertilisation (dhpf) and hours post fertilisation (hpf)

Time (dhpf)	Time (hpf)	Stage	Description
0	0	Fertilisation	
7.00	0.25	1 cell	
9.24	0.33	2 Cells	First Cleavage
11.76	0.42	4 Cells	Second Cleavage
14.00	0.50	8 Cells	Third Cleavage
17.64	0.63	16 Cells	Fourth Cleavage
24.36	0.87	32 Cells	Fifth Cleavage
33.60	1.20	Morula forms	Mulberry like stage forming
49.00	1.75	Early morula	Nuclei from the marginal cells migrate out of the cells & are distributed in the periblast
72.24	2.58	Early morula	Cells migrate into the centre of the egg
81.76	2.92	Mid morula	Cells continue to condense into the centre of the egg
88.48	3.16	Late morula	Migration into the centre continues
107.24	3.83	Late morula	Cells have merged into the centre and the blastocoel starts to form
133.84	4.78	Blastula	The embryonic shield appears indicating the onset of gastrulation
145.60	5.20	Early gastrula	The blastoderm has flattened down and follows the curvature of the yolk sphere
171.64	6.13	Mid gastrula	Epiboly progressively advances
206.64	7.38	Late gastrula	Body is just visible in the midline of the embryonic shield
211.68	7.56	Early neurula	The body is distinct in the midline
221.20	7.90	Late neurula	Tail is forming and the first pigmented cells (Melanophores) on the embryo appear
236.60	8.45	Tail-Bud embryo	Melanophores appear throughout the yolk sac
261.24	9.33	Tail-Bud embryo	Melanophores migrate to the embryo and form along the notochord
270.76	9.67	Tail-Bud embryo	Increasing pigmentation along embryo body, embryo surrounds the yolk 180^{o}
277.48	9.91	Tail-Bud embryo	9 somites visible under melanophores
284.76	10.17	Late embryo	Head continues to develop and differentiate, pigmentation along embryo is complete
298.76	10.67	Late embryo	Red blood cells circulate through the heart, Tail wrapping around the egg 180^{o}
372.40	13.30	Late embryo	Embryo surrounds the yolk 240^{o}. Eye sockets have formed
424.48	15.16	Free yolk sac larvae	Hatching begins
431.48	15.41	Free yolk sac larvae	100% larvae have hatched

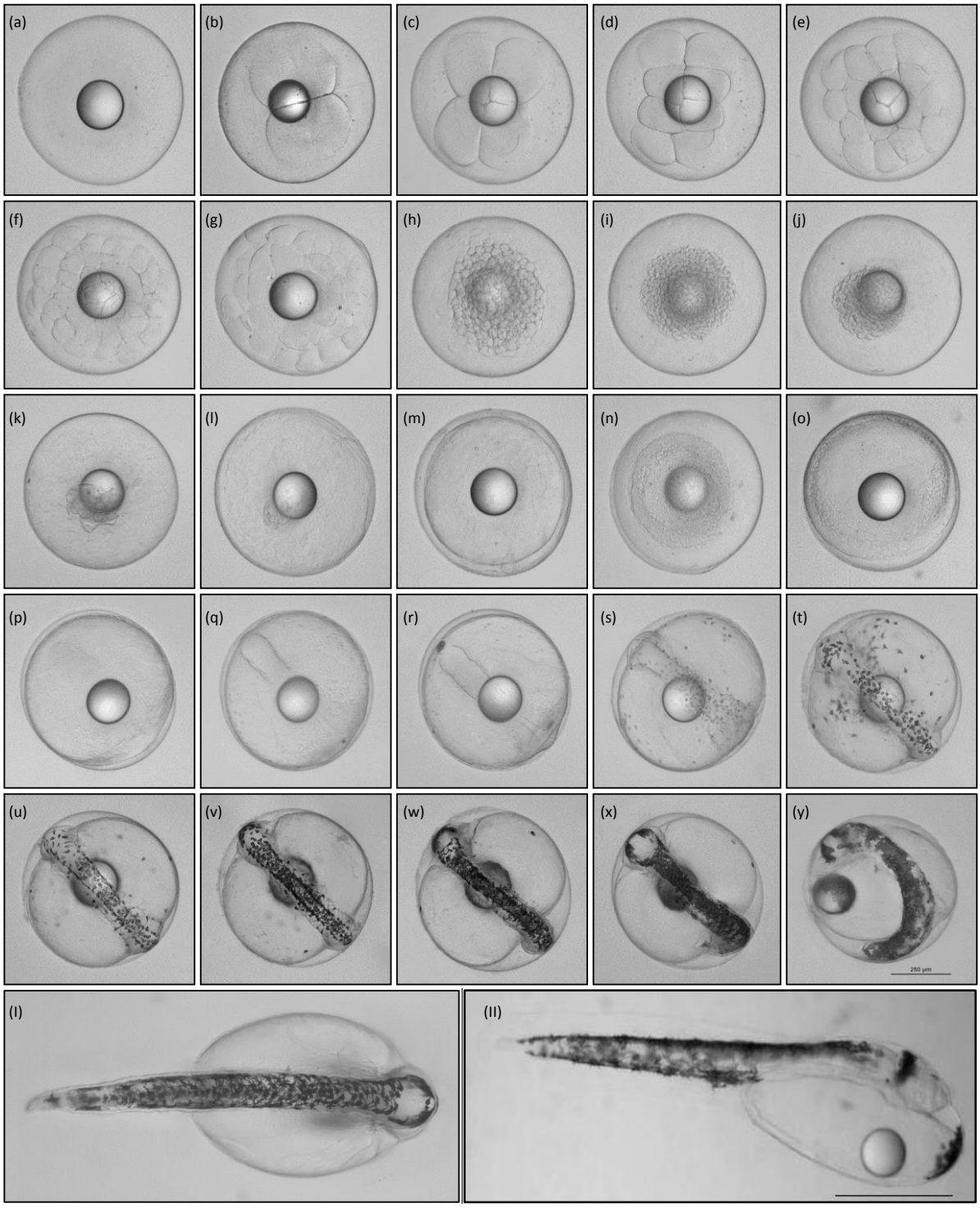

Figure 1.15: Stages of embryonic development of *K. rupestris*.

(a) fertilised egg (b) 2 cell stage; (c) 4 cell stage; (d) 8 cell stage; (e) 16 cell stage; (f) 32 cell stage; (g) morula (h) early morula stage; (i) early morula stage; (j) mid morula stage; (k) late morula stage; (l) late morula stage; (m) blastula; (n) early gastrula; (o) mid gastrula; (p) late gastrula; (q) early neurula; (r) late neurula; (s) tail-bud embryo; (t) tail-bud embryo; (u) tail-bud embryo; (v) tail-bud embryo; (w) late embryo; (x) late embryo; (y) late embryo; (I) free yolk sac larvae; (II) free yolk sac larvae. Scale bars, a–v 250 microns, I - II 450 microns.

Iodine treatment of eggs

It would appear from zero-dose controls and untreated control hatch rates that the first batch of embryos used in the iodine treatment experiments were of better quality than the second (Tables 1.16 and 1.17). The GLM of bionomial proportions for both batches of embryos was significant ($p < 0.001$) suggesting iodine treatment had an effect on hatch rate of healthy larvae. In both batches of embryos, zero-dose controls and untreated controls had better hatch of healthy larvae than eggs treated with any dose of iodine (Tables 1.16 and 1.17).

Table.1.16: Post hoc LSD test for hatching rates of embryos treated with different iodine concentrations between 0 and 100 mgL-1. Means with the same subscript are not significantly different at the P=0.050 level

Iodine treatment	Mean proportion hatched healthy larvae
Untreated control	0.8500 ab
0 mgL^{-1}	0.9588 a
25 mgL^{-1}	0.7241 b
50 mgL^{-1}	0.1043 c
100 mgL^{-1}	0.0000 d

Table 1.17: Post hoc LSD test for hatching rates of embryos treated with different iodine concentrations between 0 and 25 mgL-1. Means with the same subscript are not significantly different at the P=0.050 level

Iodine treatment	Mean proportion hatched healthy larvae
Untreated control	0.5426 a
0 mgL^{-1}	0.5653 a
15 mgL^{-1}	0.3883 b
20 mgL^{-1}	0.2604 c
25 mgL^{-1}	0.1759 c

Discussion

Latency times and fertilisation rates

The mean latency period of 55 h 21 min and the median latency period of 54 h 6 min for jungle perch induced with 1 mL Ovaprim.kg^{-1} compares favourably with the latency period of some other species of marine and freshwater fish. Average latency period for freshwater pikeperch *Sander lucioperca* injected with a dose of 600 IU.kg^{-1} human chorionic gonadotropin (hCG) was 60 h 19 min (Korbuly *et al.* 2010). The marine species, meagre *Argyrosomus regius* induced to spawn with GnRHa had latency periods ranging from 48-72 hours (Duncan *et al.* 2012).

Some catadromous species and freshwater species have shorter latency periods than jungle perch. For example, barramundi injected intramuscularly with luteinising hormone releasing hormone analogue (LHRHa) had spawning latency periods ranging from 33.7 to 47.3 h (Garcia, 1990). Australian bass *Macquaria (Percalates) novemaculeata* induced with 500 $IU.kg^{-1}$ hCG had mean latency periods of 34.2 h (Battaglene & Selosse 1996). The freshwater species silver perch *Bidyanus bidyanus* spawn 31-40 h after injection with hCG (Rowland 1984). Mean and median latency times did not vary greatly from those experienced before the commencement of the current project, but the frequency histogram from the current work has a much more prominent and narrow peak, suggesting more consistency and predictability in latency periods, which is useful for hatchery management and planning.

Fertilisation rates improved significantly in the current project compared to those experienced during pilot work. For example, during pilot work, the mean fertilisation rate was only 6% and the maximum fertilisation rate recorded (30%) was substantially lower than the mean and median fertilisation rates recorded in the current project. Improvements in fertilisation rates are most likely due to more consistent salinity levels (maintained through a recirculating biofiltration system) and more consistent temperatures around 28 ºC. There were some failed spawns with no fertilisation. In some cases this may have been related to male performance rather than egg quality. Improving male contribution to spawning could be one avenue for future research. Without the cluster of failed spawns, mean and median fertilisation rates would have been considerably higher than 56.6% and 65% respectively. A substantial number of spawns had fertilisation rates in excess of 90%. Hatcheries could therefore be expected to reliably produce large quantities of fertilised eggs.

Effects of salinity on egg buoyancy and hatch rates

It is clear from this work that it is preferential to incubate jungle perch eggs at salinities no lower than 32 ppt. Hatch rates and egg buoyancy rates are higher when the salinity is 32 ppt or higher. The best results were achieved at salinities of 36 ppt, which is the typical salinity for seawater. Unfertilised or dead eggs show a tendency to sink, so having buoyant eggs makes it easier to separate fertilised from unfertilised eggs. If salinity is maintained above 32 ppt, it is relatively easy to separate dead eggs from live eggs by swirling water in the incubation tank or egg collection bucket, and siphoning out those eggs that collect on the bottom of the tank or bucket. Tanks with a slight cone to their base assist this process.

Although some hatch can still be achieved at salinities below 32 ppt, eggs that sink to the bottom are more susceptible to bacterial infection, and the inability to separate dead eggs from live eggs can negatively impact on water quality. We recommend adding sea salt to spawning and incubation systems if the incoming seawater supply is less than 32 ppt.

The buoyancy of jungle perch eggs suggests they are marine pelagic spawners rather than estuarine spawners. (If they do spawn in the estuary, it is likely to be very close to the river mouth before any major freshwater inflow). For example, Atlantic cod (*Gadus morhua*) eggs from populations in the saline North Atlantic Basin are neutrally buoyant at 29.5-33 ppt, whereas Atlantic cod populations adapted to the less saline fjords of the Baltic sea produce eggs that are neutrally buoyant at 12.3-18.3 ppt (Jung *et al.* 2012).

In some species of fish the buoyancy of eggs can be increased by spawning fish at lower salinities because the fish increase the hydration of their eggs (May 1974; Petereit *et al.* 2009). However, this is not practical for southern stocks of jungle perch because sperm are not active enough at 30 ppt for fertilisation; maximum sperm activity was at 36 ppt and maximum duration of movement was between 34 and 36 ppt (Henderson 2010).

Egg morphometry and quality

Factors that have been reported to affect egg quality in fishes include broodstock diet and fish age (Morehead *et al.* 2001), the endocrine status of females during oocyte growth (Zarski *et al*. 2012), and husbandry practices (Bransden *et al*. 2007). Although varying egg and embryo quality may be caused by a number of these factors, egg viability generally seems to be related to morphological features observed in early development in marine fish eggs (Kjorsvik *et al.* 2003). In many cultured fish species, particularly in those new to aquaculture, unpredictable and variable reproductive performance is an important limiting factor for the successful mass production of juveniles (Izquierdo *et al.* 2001). Additionally, since early oocyte development relies on maternal mRNA and yolk proteins, eggs originated from different females may be of different quality (Brooks *et al.* 1997).

The analysis of jungle perch eggs showed mean egg diameter to be the most useful morphometric tool for assessing quality of batches of eggs as it shows a strong correlation with hatching percentage. The results support similar findings by Shields *et al.* (1997), Kjorsvik *et al.* (2003) and Rideout *et al.* (2004). Observations of egg morphology indicated little correlation with diet and or broodstock source location throughout the spawning season. These findings concur with studies for other species (Washburn *et al.* 1990; Watanabe 1993).

Since the diets that were compared showed little difference in egg morphology, there seems to be little benefit gained from providing fish with a diet high in terrestrial invertebrates, compared to the standard marine diet. Hatch data even suggest an advantage to the standard diet (see below). The shape coefficient showed that eggs were significantly more spherical in fish on the standard diet, compared to the invertebrate diet, but the shape coefficient was not a reliable indicator of egg quality as it was not correlated with egg, yolk or lipid vesicle diameter and showed no relationship with hatching percentage. However, there was little overall variation in jungle perch egg shape, with most eggs being close to spherical.

There was considerable difference between egg morphology of the ‘wild’ broodstock and domesticated broodstock (Table 1.10), even though the broodstock were maintained under apparently identical culture conditions. The eggs of the ’wild’ fish were found to have a larger mean egg diameter and lipid vesicle diameter and yolk diameter than that of domesticated jungle perch. This is in contrast to the study by Morehead *et al.* 2001, which found domesticated striped trumpeter (*Latris lineata*) eggs to have greater diameter, larger yolk volume and greater hatching percentage to that of wild fish. It is difficult to explain why this difference occurred and there were no opportunities to repeat this experiment with further wild fish. It is possible that the nutritional status of wild fish was superior and that captive diets were not optimised for jungle perch. However, the wild fish had been on captive diets for several months. The significant difference in egg quality could also be related to other environmental factors related to domestication of jungle perch. The sample size for ’wild’ fish was small compared to the number of domesticated fish used. The ’wild’ fish also tended to be smaller in size than the more domesticated fish.

There was a strong inverse relationship between number of eggs spawned and egg diameter. Larger eggs were also strongly correlated with higher hatching rates. However, number of eggs spawned was not related to the size of fish. Large fish sometimes released only relatively small numbers of eggs and on other occasions released very large numbers of eggs. Jungle perch are serial spawners with oocytes at different developmental stages during the spawning season and can spawn more than once in a season. It appears fish that release large numbers of eggs at one time allocate less resources to each egg, whereas those producing smaller numbers of eggs allocate more resources to each egg. Larger eggs may have a better chance of survival, so fewer need to be released.

Fertilisation rates and hatch rates were strongly correlated in the first spawning season, and a strong correlation between egg size and fertilisation rate was also observed. However, over the course of the project, the relationship between egg size and fertilisation rate broke down. In years two and three of the project, only three males were used in a spawning tank, whereas in year one of the project up to nine males were used in a spawning tank. The reduction in the number of males used in years two and

three may have led to reduced fertilisation by reducing the chance of a high-quality male participating in spawning. Some spawns with low fertilisation rates may have been related to male quality issues, rather than egg quality issues, leading to a breakdown in the strong correlation between egg size and fertilisation rate observed in the first spawning season. Apart from one low salinity event, from which data were discarded, water quality and temperatures were fairly consistent between years.

In the present study, the hatching rate of eggs was highly variable from 0% to 99%, with the highest values being found in the smaller 'wild' females. Higher hatching rates were also reported for eggs obtained from smaller sized gilthead seabream *Dentex dentex* (Jerez *et al.* 2011; Cejas *et al.* 2012). These results are in contrast to such species as Atlantic cod, *G. morhua* (Solemdal *et al.* 1995), Atlantic halibut, *Hippoglossus hippoglossus* (Evans *et al.* 1996) and the channel catfish, *Ictalurus punctatus* (Quintero *et al*. 2011) where hatching rate has been shown to increase with broodstock size. In the latter species larger fish may physically be more capable of devoting more resources to eggs as they increase in size. In species such as jungle perch, smaller fish may allocate more resources to individual eggs to compensate for the smaller number of eggs released.

Egg diameter and yolk diameter were strongly correlated with hatch rate, indicating that these parameters are useful in predicting egg quality in *K. rupestris*. Similar studies by Brooks *et al.* (1997) indicate the importance of egg size with regards to egg quality. Bromage *et al*. (1992) suggested that, under good hatchery conditions, differential egg size in rainbow trout, *Oncorhynchus mykiss*, is not a primary determinant of egg quality. However, as a rule, larger eggs produce larger larvae, but this does not necessarily give any permanent or long-term advantages as far as growth and survival of larvae are concerned (Kjorsvik *et al*. 1990). Rainbow trout larvae are far larger than jungle perch larvae and therefore perhaps less restricted in food choices. However, in the case of small pelagic larvae like those of jungle perch, slightly larger larvae should have an advantage by being able to feed on a wider choice of plankton species. Very small larvae may be more restricted in the number of copepod or other species they can prey on.

The number of eggs spawned over the season may be highly variable within populations (Trippel 1998). In communal broodstock spawning, such as was the case with jungle perch, each spawning may include eggs from more than one female. Differences in egg quality parameters because of parental variation may be high (Bromage 1995). Furthermore, with multiple females in a communal tank, there is no way to determine the relative contribution of viable and unviable eggs from individuals (Aristizabel *et al.* 2008) apart from genetic analyses (Hoskin *et al.* 2015). The results show a pattern of highly variable inter-population relationships among morphological parameters in communally spawning jungle perch broodstock populations.

Biochemical composition of eggs

This study represents the first investigation into the quality and biochemical composition of jungle perch eggs. The results provide baseline information regarding the composition and quality of jungle perch eggs spawned by broodstock on a standard diet that will be useful for future comparisons with formulated feeds. Egg quality and composition can be affected by broodstock diets (Izquierdo *et al.* 2001). The biochemical composition of fish eggs is thought to be one of the main determinants of egg quality due to the primary influence of the biochemical constituents on fish development (Kamler 2005). Biochemical composition of eggs is often used as an indicator of egg quality, but the relationship between biochemical composition and egg quality is difficult to interpret (Morehead *et al.* 2001). Each of the biochemical components in the egg have different roles and the relative importance of each component varies as the requirements for each nutrient change throughout egg development (Wiegand *et al.* 2004).

For jungle perch eggs, proportions of dry weight, moisture content, total lipid and total protein were fairly consistent across diets and no trends were observed over the course of the spawning season. Egg dry weight and moisture content vary among species and has been shown to either vary or remain relatively constant during the spawning season. Changes can be due to seasonal changes in environmental parameters, asynchronous spawning of different sized females or depletion of maternal

resources (Kamler 2005). For example, Chambers & Waiwood (1996) reported egg size declined over the spawning season for Atlantic cod (*G. morhua*) and attributed the decline to decreased feeding activity by adults during spawning and subsequent reduction of maternal nutrition. Unlike Atlantic cod, jungle perch feed continuously during the spawning season helping to maintain condition of the adults during the spawning season.

In the present study, the volume of total protein and total lipid in the eggs of the jungle perch was observed to be higher for those from fish on the invertebrate diet than for eggs from fish on the standard diet, but the differences were not statistically significant. Total protein content of the jungle perch eggs ranged from 48% to 54% with the mean protein content at 50%. This is lower than the results obtained for two Australian freshwater species, the Macquarie perch (*Macquaria australasica*) (65%) and the trout cod (*Maccullochella macquarensis*) (70%) (De Silva *et al.* 1998). Total protein content in marine species, such as Atlantic cod (*G. morhua*) (36%) (Lanes *et al.* 2012) and cobia (*Rachycentron canadum*) (25%) (Faulk and Holt 2008), can be considerably lower than that of freshwater species. These differences in protein levels may be related to the amount of total amino acids in marine fish eggs present as free amino acids which can be up to 50% (Rønnestad *et al.* 1999). Total protein content of catadromous jungle perch eggs appears to sit at a value intermediate between marine and freshwater species.

Free amino acids are the main energy resources during egg development; they also are osmotically active compounds as they regulate egg hydration and subsequently egg buoyancy during final oocyte maturation (Rønnestad *et al.* 1992; Rønnestad *et al.* 1998). Several studies have demonstrated that free amino acids are lower in nonviable than in viable eggs (e.g. Nocillado *et al.* 2000; Maeland *et al.* 2003). There was no significant difference observed in the total amino acid content across diet treatments in jungle perch although there was considerable variation within both diet treatments throughout the 2012/2013 spawning season. The current study was unable to look at individual amino acid composition in jungle perch eggs. A more detailed breakdown looking at amino acid composition and comparing amino acid composition and egg quality parameters may provide further insight to jungle perch nutritional requirements to maximise egg quality.

Lipids and fatty acids, especially essential fatty acids (EFA), in the broodstock diet greatly affect egg composition and subsequent egg and larval quality (Izquierdo *et al.* 2001). Numerous studies have confirmed the importance of an adequate supply of lipids, and particularly n-3 HUFA in the diets of fish used as broodstock in aquaculture (Watanabe, 1993). This has been particularly important during the process of gametogenesis (Sargent 1995, Bell *et al.* 1997). According to Sargent (1995), fatty acid composition of eggs is generally more conserved and relatively less influenced by the diet than other fish tissues, reflecting the importance of the specific composition of gametes. Nevertheless, many studies have shown that egg fatty acid composition reflect those in the broodstock diets of both marine and freshwater species (Furuita *et al.* 2002; Furuita *et al.* 2006; Zakeri *et al.* 2009; Henrotte *et al.* 2010). This accords with the current study that demonstrated the broodstock diet closely reflecting the composition of the oocytes.

The total lipid content of the eggs of two Australian freshwater species the Macquarie perch (*M. australasica*) and trout cod (*M. macquarensis*) has been found to range from 15% - 20% (De Silva *et al.* 1998) which is consistent with the total lipid content of jungle perch eggs in this study. Marine fish eggs have also been reported to range from 15–35% total lipid content (Vetter & Hodson 1983; Ostrowski & Divakaran 1991; Sargent 1995; Dayal *et al.* 2003). In the case of crude lipids, there are no reported studies on the optimum crude lipid requirements of marine broodstock. Requirements for egg production probably vary from those required for optimal growth as most of the nutrients that affect egg quality are transported to the eggs via lipid micelles during gonad maturation (Sargent 1995). Lipid and fatty acid composition of eggs of Atlantic halibut (*H. hippoglossus*) are different between repeated and first-time spawning broodstock (Evans *et al.* 1996). These latter results suggest that egg composition is probably affected by the broodstock physiological condition, as well as broodstock nutrition.

Analysis of the jungle perch diet by Pusey *et al.* (2004) identified terrestrial invertebrates such as ants, spiders, cockroaches and crickets comprising a large component of the jungle perch diet in Cape York Peninsula. Based on the analysis by Pusey *et al.* (2004), the current study incorporated a diet with a high terrestrial invertebrate component as an alternative to a more standard diet consisting mostly of pellets and marine derived feeds, although neither diet was exclusively marine or terrestrial. The main components in the invertebrate diet consisted of common meal worm larvae, giant mealworm larvae, black soldier flies and blowfly larvae.

Analysis of the terrestrial diet and marine diet components used in this study has been reported elsewhere in the literature. Fatty acid profiles of the key marine and terrestrial dietary components are shown in Table 1.18. High levels of the mono-unsaturated Oleic acid (18:1n9) are found in common meal worm larvae (Finke, 2002), giant mealworm larvae (Finke 2002), black soldier flies (St-Hilaire *et al.* 2007) and blowfly larvae (Guarnieri *et al*. 1976), whereas the standard diet consisting of prawns (Ouraji *et al.* 2011), pilchards (Guil-Guerrero *et al.* 2011) and green lip mussels (Chan *et al.*, 2003) has significantly higher levels of the HUFAs Eicosapentaenoic acid (EPA, 20:5n3) and Docosahexaenoic acid (DHA, 22:6n3). Australian freshwater species, such as Murray cod (*M. peelii peelii*) (Turchini *et al.* 2006), Macquarie perch (*M. australasica*) (De Silva *et al.* 1998) and trout cod (*M. macquarensis*)(De Silva *et al.* 1998), are known to thrive on diets containing only C_{18} mono- and polyunsaturated fatty acids as the source of essential fatty acids.

Table 1.18: Fatty acid profiles across diet components (percentage)

Fatty acid profile	Giant mealworm (1)	Common mealworm (1)	Blowfly larvae (2)	Black soldier fly (3)	Prawns (4)	Pilchards (5)	Green lip mussels (6)
Myristic acid (14:0)	<0.1	<0.1	<0.1	2.9	1.6	5.8	2
Palmitic acid (16:0)	15	18	11	16	19.2	23.7	12.1
Stearic acid (18:0)	<0.1	<0.1	0.5	5.7	10.6	6.4	1.5
Oleic acid (18:1n9)	39	43	29.4	32.1	13.6	10.6	0.4
Linoleic acid (18:2n6)	49	28	12.9	4.5	7.3	1.6	0.5
Linolenic acid (18:3n3)	<0.1	<0.1	14.7	0.2	1.3	0.9	0.3
Arachidonic acid (20:4n6)	<0.1	<0.1	<0.1	<0.1	4.7	0.7	2
Eicosapentaenoic acid 20:5n3	<0.1	<0.1	<0.1	<0.1	12.4	9.1	12.3
Docosahexaenoic acid 22:6n3	<0.1	<0.1	<0.1	<0.1	8.8	11.8	24.4

(1) Finke (2002) (2) St-Hilaire *et al.* (2007) (3) Guarnieri *et al.* (1976) (4) Ouraji *et al.* (2011) (5) Guil-Guerrero *et al.* (2011) (6) Chan *et al.(* 2003)

The situation is different with marine species where it is known that these species require dietary long-chain n-3HUFA such as EPA and DHA for optimal growth and survival (Sargent *et al.*, 2002). A nutritional trial by Tocher & Sargent (1987) implicated deficiencies in the fatty acid desaturation-elongation pathway as being central to the dietary requirements for HUFA in marine fish. This was supported by a cell culture survey dissecting HUFA synthesis in fish using radiolabelled fatty acid substrates for individual desaturation and elongation steps. This identified primary deficiencies in the pathway at the Δ5 desaturase in a marine sea bream cell line (Tocher & Sargent, 1987).

The fatty acid requirements of jungle perch broodstock is not yet known; however, there was a significant difference in the fatty acid composition of eggs produced across diet treatments. In the present study, a significant proportion of C_{18} PUFA and C_{18} monounsaturated fatty acid was found in eggs of broodstock fed on the invertebrate diet. This indicated the presence of terrestrially derived oils in their feeds and potentially the low capability of the fish to bio-convert this fatty acid into $C_{20/22}$

HUFA. If this is the case, then the catadromous jungle perch is more like a marine species than a true freshwater species in this respect. Deficiency of n-3 highly unsaturated fatty acids has been found to depress egg quality of several species (Watanabe 1993; Furuita *et al.* 2002; Furuita *et al.* 2006). This was also evident in the current study with hatching rate significantly negatively correlated with SFA content. Fish eggs that contain a high level of n-3 HUFA, in particular DHA, have an important role in embryonic development as well as at the larval stage (Tocher *et al.* 1985; Furuita *et al.* 2000; Furuita *et al.* 2002).

Terrestrial insects may be an important component of wild jungle perch diet and probably contribute to growth and protein requirements, but may not be significant for development of egg quality. It is likely that the aquatic components of the wild jungle perch diet, including fish, crustaceans and aquatic insects (Pusey *et al.* 2004) may be more important for the development of high quality eggs. It is not known if jungle perch feed in the estuary on their way to spawning sites. If they do it may also help contribute to egg quality.

The n-3 HUFA is one of the most important group of nutrients in the broodstock diet of marine species (Bell *et al.* 1997; Furuita *et al.* 2002; Furuita *et al.* 2006). DHA, in particular, has a high biological value during larval development (Watanabe 1993; Furuita *et al.* 2002; Furuita *et al.* 2006; Trushenski *et al.* 2010). Results of this study strongly suggest that DHA n-3 HUFA have an important role for jungle perch egg and larval development as observed in other species. DHA was significantly positively correlated with hatching rates.

Studies have suggested that dietary fatty acid can markedly affect the overall lipid metabolism (Sargent *et al.* 1999; Sink *et al.* 2010; Lanes *et al.* 2012), and in general, there was an indication that freshwater fish fed C_{18} PUFA-rich vegetable oils were capable of $C_{20/22}$ HUFA production. However, the range of the desaturase activity measured using ex vivo methods is quite considerable and variable between different species, and even within a species (Zheng *et al.* 2004). The principal observation reported here is that jungle perch may not be able to elongate and desaturate? dietary linoleic and α-linolenic acids. They probably lack the required elongase, Δ6 and Δ5 desaturase, activities. For maintenance of jungle perch broodstock it would appear best to treat them like marine broodstock to obtain the highest quality eggs.

The mean fertilisation rate was significantly higher for the standard diet treatment than in the invertebrate diets. This was possibly due to elevated dietary levels of HUFA found in the standard diet compared with the invertebrate diet. A similar trend although not significant, was reported in the hatching rate from the standard diet compared to the invertebrate diet. High variability in fertilisation success during induced spawning of aquaculture species is common and is likely a result of many interacting factors including stress, egg over-ripening, sperm quality and hormone dose (Bromage *et al.* 1992 ; Bromage 1995). It would appear for jungle perch broodstock, that diet is one factor that can affect fertilisation rates.

Based on the apparent inability of the jungle perch to convert C_{18} PUFA into $C_{20/22}$ HUFA it can be inferred that the recent ancestors of jungle perch were marine fish that evolved to become a catadromous species that lives predominantly in freshwater. They have retained their requirement to spawn at sea, milt being non-motile in fresh water (Hogan and Nicholson 1987). Production of large numbers of very small (0.7 mm) pelagic eggs by jungle perch is typical of many marine fish (Giménez *et al.* 2006). For example, marine species such as pink snapper (*Pagrus auratus*) (Fielder *et al.*, 2005) and gilthead sea bream (*D. dentex*) (Lahnsteiner *et al.* 2009), have oocytes reaching 1 mm diameter fully hydrated. In contrast to jungle perch, many fresh water species such as the salmonids (*Salmo trutta*), can have eggs as large as 5 mm in diameter (Sahini *et al.* 2007). Sooty grunter (*Hephaestus fuliginosus*) is a fish that occupies a similar niche to jungle perch in freshwater, and it co-occurs with jungle perch in north Queensland. This freshwater spawner has water-hardened eggs with a mean diameter of 2.1 mm (Hogan 1990). This is three times the diameter of jungle perch eggs and 27 times the volume. However, some freshwater spawners such as spangled perch (*Leiopotherapon unicolor)* do produce small eggs of similar size to jungle perch (Pusey *et al*. 2004).

Embryonic development

Jungle perch eggs display meroblastic cell division and follow the general pattern of embryogenesis observed in other teleosts (Friogeirsson 1978; Cetta & Capuzzo 1982; Gosh, 1985; Westernhagen *et al.* 1988; Pickova *et al.* 1997; Bermudes & Ritar 1999). The eggs are positively buoyant, spherical and hatch into yolk-sac larvae. They were centrolecithal, having a chorion and translucent, homogeneous yolk. The eggs are transparent, non-ornate and lack appendices, all features typical of pelagic eggs (Ahlstrom & Moser 1980; Westernhagen *et al.* 1988; Pauly & Pullin 1988; Rønnestad *et al.* 1996; Moran *et al.* 2007). Additionally, the undifferentiated newly hatched larvae have an unopened mouth and eyes lacking pigmentation (Carton & Vaughan, 2010), which is typical of the early life stages of pelagic fish larvae (Llewellyn 1973; Johannes 1978; Ahlstrom & Moser 1980; Pauly & Pullin, 1988).

In the present study, six embryonic developmental stages were described: fertilisation, blastodisc cleavage, gastrulation, organogenesis, pre-hatching and hatching. Each of these stages is characterised by distinct morphological and physiological features (Blaxter 1969; Friogeirsson 1978). Like many other teleost species, jungle perch has blastomeres of regular size and shape. The first five cleavages divided into 32 equal-sized blastomeres at the animal pore before the blastodisc attained the berry-like appearance of the morula. The start of the basic embryonic tissue stage is identified by the blastoderm, which resembles tissue rather than a grouping of individual cells. Gastrulation continues by proliferation of cells around the germ ring, a process known as 'epiboly'. The start of organogenesis is characterised by the development of organs, during which systems of organs become functional. Preparing for hatching marks the end of organogenesis. This is the last stage before hatching and is characterised by the elongation of the embryo reaching up to three quarters of the eggs circumference.

Jungle perch larvae started hatching after 15.16 h at 28 °C (424.5 degree hours post-fertilisation (dhpf) and the entire batch of eggs was fully hatched by 15.41 hours (431.5 dhpf). Tropical marine fish that have small eggs, combined with the high temperatures of their habitat generally have extremely short egg development times (Polovina & Ralston 1987; Pauly & Pullin 1988; Kjorsvik & Holmefjord 1995), which is consistent with the results obtained in this study. Therefore, jungle perch can be characterised as a typical tropical marine pelagic spawner. Other marine-spawning, pelagic species that have spherical eggs similar to that of jungle perch with similar hatching times include: bigeye scad *Caranx crumenophthalmus* 0.78 mm, 423.26 dhpf, shortfin scad *Decapterus kurra* 0.70 mm, 342 dhpf and silver perch *Bairdiella chrysoura* 0.76 mm, 495 dhpf (Pauly & Pullin 1988).

The mangrove jack *Lutjanus argentimaculatus*, has a similar life history to jungle perch, in that it uses freshwater habitats for maturation and spawns in marine environments (Russell *et al.* 2003). Mangrove jack have transparent, positively buoyant pelagic eggs with mean egg diameters of 0.82 mm and an incubation time of 18h 10 min at 29.0 ºC (Cowden 1995). Jungle perch eggs in comparison are smaller (0.660 mm) and develop more quickly. The euryhaline yellowfin bream *Acanthopagrus australis* spawns in winter and early spring. It also has buoyant, transparent pelagic eggs with a mean size of 0.79 mm and an incubation period of 22.5-44.2 h over a temperature range of 19.4-27.7 ºC (Cowden 1995).

Barramundi *Lates calcarifer* is another species with a similar catadromous life history to jungle perch, except that barramundi can spawn at lower salinites. Barramundi spawn in estuaries at salinities between 17 and 31 ppt (Allen *et al.* 2002). Newly released eggs of the diadromous barramundi were found to have a mean diameter of 0.796 mm (Thépot & Jerry 2015). Time to hatch for barramundi eggs was 15 h 51 min, 11h 57 min, 10 h 36 min and 10 h 20 min for eggs incubated at 28, 30, 32 and 34 ºC respectively.

The present study has produced a description of embryonic development in jungle perch together with the timing of key developmental milestones. This study provides baseline data that is needed to better understand the lifecycle of jungle perch. In general, jungle perch eggs are smaller and develop faster at equivalent temperatures than most of the pelagic spawners that have been reviewed here. Understanding normal larval development can provide a basis for a better understanding of the egg quality and reproductive development of this species in captivity.

We have found that yolk sac larvae and early stage larvae of jungle perch are quite sensitive to handling. We consider it best to move late stage embryos rather than just hatched larvae to larval rearing tanks or ponds. If transferring embryos to ponds or rearing tanks, this is best done between 10 and 14 hours after fertilisation at incubation temperatures of 28 ºC. Turning off aeration and water supply in the incubation tank leads to most embryos rafting on the surface after approximately 10 minutes, whereas dead eggs will tend to sink to the bottom. Rafting embryos can be skimmed from the surface for transfer to rearing tanks or ponds (see Chapter 2). This should lead to better survival than transferring hatched larvae.

Treating eggs with iodine

Results for iodine treated grouper *Epinephelus coioides* eggs showed that Vibrios were eliminated by iodine disinfection at concentrations between 2.5 ppm and 20 ppm applied for 10 min (Tendencia 2001). Iodine concentrations of 15 ppm and 20 ppm applied for 10 min were effective at significantly reducing total bacterial load, but hatching rates were also significantly lower (Tendencia 2001). Similarly, hatch rates of jungle perch eggs in this project were significantly reduced at iodine concentrations of 15 $mg.L^{-1}$ and 20 $mg.L^{-1}$, concentrations that could reasonably be expected to effectively reduce bacterial load. In contrast, a dose of 100 ppm iodine applied to the morula developmental stage for 10 minutes was effective at disinfecting Black Sea turbot *Psetta maxima* eggs without adversely affecting hatch rates (Aydin *et al.* 2011). A 100 ppm dose of free iodine for 10 minutes is standard practice for treating trout (salmonid) eggs in the Pacific Northwest region of the USA (Hinshaw 1990).

For the ornamental freshwater fish species black tetra *Gymnocorymbus ternetzi*, a dose of iodine of 1 $mg.L^{-1}$ applied for the entire incubation period (24 h), improved hatch rates. However, no concentration of iodine applied over the incubation period of zebrafish *Danio rerio* (24 h) or angelfish *Pterophyllum scalare* (48 h) was found to improve their hatch rates (Chambel *et al.* 2014). A dose of iodine of 1 $mg.L^{-1}$ is less than any concentration tested on jungle perch eggs. The higher doses tested on jungle perch were applied for a much shorter time period.

Jungle perch eggs appear sensitive to iodine even at doses lower than those used as a standard 10 min treatment for eggs of various other species of fish species including salmonids. Doses less than 15 $mg.L^{-1}$ were not tested on jungle perch eggs. It might be worth examining longer exposure times (*e.g*. full incubation period) at very low doses of iodine such as 1$mg.L^{-1}$to determine whether the benefits observed by Chambel *et al.* (2014) with *G. ternetzi* may apply here . Other potential disinfection methods that could be tested in the future include ozonation of eggs and hydrogen peroxide treatment (Lahnsteiner et al. 2009).

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Chapter 2: Optimising larval and juvenile rearing

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Summary

Past rearing attempts for jungle perch *Kuhlia rupestris* larvae using green-water rearing systems with the microalga *Nannochloropsis oculata* and provision of either rotifers or super small (ss)strain rotifers as a first feed, failed to result in any larval feeding and all larvae starving to death by six days post hatch when reared at 26 ºC. In this chapter we describe a range of larval rearing experiments where the effect of temperature, lighting, salinity, tank background colour, larval feeds, tank size and aeration systems and pond rearing conditions were all examined.

A temperature of 28 ºC was a suitable temperature for rearing jungle perch larvae. Nauplii of the copepod *Parvocalanus crassirostris* (reared with the microalga *Isochrysis* aff *galbana* T-Iso) were found to be a suitable first feed for jungle perch larvae. Feeding and larval survival were significantly improved by provision of bright light (2500 lux) over tanks using a 16 h light and 8 h dark photoperiod. This lighting regime also improved swim-bladder inflation. It would appear that feeding is essential prior to swim bladder inflation, to enable jungle perch larvae to reach a sufficient size to break through the surface tension to inflate their swim-bladders.

There was some evidence for higher rates of swim bladder inflation in large tanks (diameter 3.1 m) compared to small tanks (diameter 1.1 m), but high levels of swim bladder inflation could still be achieved in small tanks if copepod nauplii densities were high. Survival of larvae in small tanks was not significantly different to that of larvae in large tanks.

Tank background colour (blue, black, yellow and white) had no significant effect on larval survival. However, in a comparison of granite and blue coloured backgrounds, better survival was achieved in tanks with blue backgrounds. Larvae in tanks with upwelling systems had poor survival compared to larvae reared in lightly aerated tanks. Upwelling appeared to prevent larvae from feeding efficiently. Larval survival was higher in tanks with a salinity of 35 ppt (full seawater) than in tanks with a salinity of 28 ppt. This may be related to reduced buoyancy of yolk sac larvae at lower salinities. Larvae that remain near the bottom in lower salinities may be more susceptible to bacterial infection.

Larvae could not be successfully weaned from copepod nauplii and copepod diets to ss strain rotifer or rotifer diets. The capacity to produce large numbers of copepods and T-Iso at BIRC was limited, therefore long term rearing in tanks with the T-Iso and *P. crassirostris* production system was not practicable. An alternative approach was to rear larvae through to the fingerling stage in ponds. This was achieved. Larvae could either be stocked into ponds as yolk sac larvae or embryos, or stocked into ponds after one week of tank rearing. The duration of copepod nauplii blooms (above 50 nauplii.L^{-1}) in ponds was significantly and positively correlated with the number of fingerlings produced. From 20 days post hatch feeding of larvae in ponds could be supplemented with *Artemia* nauplii and commercial weaning diets suitable for barramundi larvae.

Jungle perch larvae aggregate near the pond surface for feeding. Therefore, paddlewheels should not be used for aerating ponds. We found a system of air-stones and airlifts produced adequate aeration without harming larvae. After metamorphosis jungle perch sit deeper in the water column.

Jungle perch larvae begin feeding three days post hatch. Swim bladder inflation occurs between four and seven days post hatch. Metamorphosis occurs when larvae are 16 to17 mm total length (TL). Depending on growth rates this may occur from 28 to 34 days post hatch. Larvae are robust enough for harvesting and transfer to freshwater at 25 mm TL, which is usually between 50 and 60 days post hatch.

Introduction

Preliminary work on jungle perch rearing prior to this FRDC project by the DAF research team had failed to rear any jungle perch past 5 days post-hatch at 26 ºC. The preliminary work had tried rearing jungle perch larvae in light blue tanks, in full seawater, with lightly aerated green-water (*Nannochloris*) cultures containing super small (ss) strain rotifers held at ambient light under a shade-cloth covered translucent roof. There was no evidence of feeding and it appeared that larvae had starved to death. There was some indication that ss strain rotifers may not have been a suitable feed, as larvae stocked into a pond with a stocked ss rotifer culture had also failed to develop. However, whether the tank rearing (and pond rearing) trials had failed because of the choice of food available or other factors related to rearing conditions, such as temperature, lighting, aeration regime, or salinity remained unknown. It was clear that further research was required in these areas and thus larval rearing conditions and larval feeds became an area of focus for the current research project. This included both tank- and pond-rearing of larvae.

As noted in the previous chapter, jungle perch produce small pelagic eggs and newly hatched yolk sac larvae are small (1.8 ± 0.2 mm in length). Given the small size of jungle perch larvae, choice of the appropriate first feed could be critical. Cowden (1995) had similar difficulties in getting mangrove jack larvae *Lutjanus argentimaculeatus* to survive when offered a range of feeds, including screened rotifers, oyster trochophores and screened wild zooplankton. Over 95% of mangrove jack larvae died between days 3 and 6 post hatch, corresponding with transition to exogenous nutrition. Complete mortality was observed by day 12. Cowden (1995) concluded that the cause of mortality appeared to be starvation, but believed it may have been a secondary consequence of sub-optimal egg quality, physical rearing conditions or inappropriate initial food items. According to Rimmer (1998), mangrove jack larvae in Taiwan are reared outdoors in concrete or earthen ponds and are stocked into ponds as eggs. Larvae are held initially in tarpaulin enclosures set in the pond, to which oyster trochophores are added as a feed. Two days after first feed, larvae are released into the pond. Rotifers and other zooplankters cultured in small concrete or earthen ponds are added to the main pond as larval feeds. These feeds are no different to those trialled by Cowden (1995), but the rearing conditions were different.

First feed choice is normally dictated by larval mouth size (Kraul 2006), who noted that small-mouthed fish larvae may require feeds smaller than juvenile rotifers. Examples include peacock grouper *Cephalopholis argus,* flame angelfish *Centropyge loricula* and snappers *Pristipomoides filamentosus*. The latter will ingest rotifers but does not survive well unless fed copepod nauplii (the larval stage of copepods). Peacock grouper require copepod nauplii initially, but grow large enough to take rotifers after a few days feeding. The angelfish requires copepod nauplii for at least 10 days. Kraul (2006) also noted that even smaller feed items, such as the ciliate *Euplotes*, can be cultured and are associated with the successful rearing of some fish larvae. *Euplotes* have been found in the gut of first feeding larval grouper (Nagano *et al.* 2000). Rhodes & Phelps (2006) found a combination of the ciliated protozoan *Fabrea salina* with copepod nauplii as a first feed improved survival of red snapper *Lutjanus campechanus* larvae. Combination of oyster trochophores with ss type rotifers improved survival of Nassau grouper *Epinephelus striatus* larvae compared to rearing on just rotifers alone. Oyster trochophores were inadequate when used exclusively (Watanabe *et al.* 1996). Therefore

combinations of feeds or sequencing of feeds may be important to improve survival of fish larvae, including jungle perch.

Copepods form a major component of the natural diet of many fish larvae. The wide range of body sizes both within and between species makes them extremely useful for employing copepod nauplii and copepodites as a starter feed for small larvae. Improved survival of several species of finfish larvae has been noted when they are reared on copepods. This has generally been attributed to levels of the fatty acids Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) and particularly to the DHA: EPA ratio in the diet (Gopakumar & Santhosi 2009).

It may not always be size that limits the first prey choice of some species of fish larvae (Ostrowski & Laidley 2001). It could also be swimming behaviour of the prey or other environmental or behavioural cues. Young (1994a,b) noted that the larvae of some marine species refuse to eat rotifers (s-type) even though the larvae are large enough to catch them. Super small type rotifers offered to jungle perch larvae in pilot work by the DAF research team were considered small enough to be consumed by jungle perch larvae, but the larvae did not exhibit any feeding behaviour. Moe (1997) considered the stop and go motion of copepod nauplii was needed to initiate predatory behaviour in *Pomacanthus* species compared to the simple whirling motion of the rotifer. Buskey (2005) and Marcus (2005) also attributed the jerky swimming motion of copepod nauplii and adults to stimulating feeding behaviour. Schipp (2006) noted that golden snapper *Lutjanus johnii* and peacock grouper *C. argus* require copepods to successfully negotiate past first feeding, whereas other species such as the flame angelfish *C. loricula,* appear to need copepods for the entire larval life cycle.

Prior to the completion of the current project on jungle perch *K. rupestris,* the only successful rearing of a *Kuhlia* species was reported for *K. mugil,* a fully marine species. Information was limited and reported in a blog linked to the Long Island Marine Aquarium (Gardner 2013). Overall survival of *K. mugil* larvae was poor but a small number of larvae were successfully reared through to metamorphosis. First feeding larvae readily accepted nauplii of the copepod *Parvocalanus crassirostris* and were fed a diet of copepods (*Parvocalanus*, *Acartia* and *Pseudodiaptomus*) and rotifers throughout the larval period before weaning onto Cyclop-eeze® and *Artemia*.

Other than feeds, rearing conditions can also impact on the success of rearing fish larvae. A range of environmental variables can interact to influence larval survival. Temperature can affect the feeding ability of larval fishes (Paul 1983; Johnson & Katavic 1986; Batty & Morley 1994). There are temperatures at which feeding is more efficient. Temperatures that are too high or too low may inhibit feeding. For example, witch flounder *Glyptocephalus cynoglossus* did not survive beyond first feeding if reared at temperatures of 5.1, 10.4 or 19.5 ºC. Maximum first feeding occurred between 15 and 16.2 ºC (Bidwell & Howell 2001).

There was no evidence of swim-bladder inflation in jungle perch larvae during pilot studies by DAF staff. For tank-reared larvae, surface skimmers to keep the tank surface clear of lipids and protein scum can facilitate swim-bladder inflation in larval fish (Fielder & Heasman 2011; Moran *et al.* 2011). Lighting intensity and photoperiod can also influence swim-bladder inflation, feeding efficiency and survival. For example, some larvae may need to feed in order to grow to a size large enough to push through the surface tension to inflate their swim-bladders. The light threshold levels at which pollock larvae capture prey is between 0.2 and 0.4 lux (Paul 1983). In contrast survival of yellowtail kingfish *Seriola lalandi* was much higher at 32 000 lux than at 1000 lux (Woolley *et al.* 2012), whereas cod *Gadus morhua* had most success at foraging when reared under 2200 lux from day 3-27 then at 600 lux from day 28-58 (Monk *et al.* 2006).

In yellowtail kingfish, swim-bladder inflation was significantly higher for those reared under an artificial light source than under natural lighting (Woolley *et al.* 2012). In contrast, Australian bass *Macquaria* (*Percalates*) *novemaculeata* were found to have higher swim-bladder inflation rates under conditions of darkness with low aeration and a salinity of 25 ppt. Inflation rates were reduced under 12 h dark 12 h light conditions. There were also interactions between salinity levels and aeration conditions. Inflation was reduced under high aeration and at a salinity of 10 ppt (Battaglene & Talbot

1990). Under zero light conditions (24 h dark) greater amberjack larvae *Seriola dumerili* had total mortality eight days after hatching. From 12 h to 24 h light conditions there was no significant difference in swim-bladder inflation or larval feeding. Survival and growth were best under an 18 h light and 6 h dark photoperiod and larval survival was highest at 22 ºC (Hirata *et al.* 2009).

For some species tank background colour has been shown to make a difference to larval survival. Some tank colours may assist fish larvae to visualise prey by providing greater contrast. Some coloured backgrounds may be more or less stressful to fish (McLean *et al.* 2008). Juvenile tilapia and flounder maintained in red coloured tanks returned better per cent increases in weight and had lower plasma cortisol levels than fish maintained in black, green, dark blue or light blue tanks (McLean *et al.* 2008). Opiyo (2010) found Nile tilapia *Oreochromis niloticus* fry had better growth and survival in blue background tanks, compared to clear background and black background tanks.

Dolphin fish *Coryphaena hippurus* larvae had higher survival in black tanks than tan coloured tanks. Rotifer density had an effect on survival of *C. hippurus* larvae in tan tanks and was highest at 20 rotifers.mL^{-1}. In black tanks there was no difference in *C. hippurus* larval survival rates at rotifer densities in the range of 5-20 mL^{-1}. This suggests feeding efficiency was improved in the darker tank. In a contrasting result, thinlip mullet *Liza ramada* larvae fed on an experimental artificial diet achieved the highest growth rates and survival in light coloured (clear, white or yellow) tanks. Fish held in dark coloured tanks (red, green, black and blue) did less well. Use of dark coloured feeds in light coloured tanks further improved growth and survival (El-Sayed and El-Ghobashy 2011).

Effects of light intensity and tank background colour have also been studied for Eurasian (redfin) perch *Perca fluviatilis* larvae. Out of white, light grey, dark grey and black tank background colours, survival was best in light grey tanks. The effect of light intensity was more complex. Survival was best at 250 lux, but growth was best at 800 lux (Tamazouzt *et al.* 2000).

From the above references it is clear that lighting and tank background colour can influence the growth and survival of fish larvae, but the optimum levels of light and tank colour vary between species and feed types. Tank backgrounds can also influence walling behaviour in fish. Walling is when fish larvae actively swim into tank walls. This can lead to jaw damage (Cobroft & Battaglene, 2009) which in turn can lead to poor feeding performance and reduced survival. Walling has been explained as a photo-positive attraction to the tank wall (Bristow & Summerfelt 1994; Naas *et al.* 1996).

Naas *et al.* (1996) suggested black tanks would reduce walling behaviour. Cobroft & Battaglene (2009) experimented with different tank background colours to reduce walling behaviour in striped trumpeter *Latris lineata*. Walling behaviour was reduced in tanks with black or with marble (speckled black, grey and white) backgrounds. The highest proportion of jaw damage occurred in red tanks, followed by green, white, blue, black and then marble. Growth and survival were highest in the black tanks. Some success in reducing walling behaviour and increasing survival of yellowtail kingfish *S. lalandi* has been achieved in tanks with granite (speckled) backgrounds (Yeoman 2014).

Jungle perch are a catadromous species. Optimal salinities for rearing catadromous and estuarine fish larvae vary considerably between species. Mulloway *Argyrosomus japonicus* larvae have higher survival between 5-10 ppt than at higher salinities (Fielder & Heasman 2011). Australian bass *M.*(*P.*) *novemaculeata* larvae are best reared at 15-35 ppt (Fielder & Heasman 2011). Catadromous Japanese eel *Anguilla japonica* larvae are best reared at 34 ppt at 25 ºC (Kurokawa *et al.* 2013). Rearing salinities can alter nutritional requirements of fish larvae. For example, larvae of the catadromous *Galaxias maculatus* require lower levels of ω-3 highly unsaturated fatty acids (HUFA) in their diet for optimum growth if reared at 15 $g.L^{-1}$ salinity compared to conspecifics reared at 0$g.L^{-1}$ salinity (Dantagnan *et al.* 2013).

Variations in aeration rate or use of upwelling in tanks have also been used to improve larval survival. There is a peak in mortality of yellowtail kingfish during the mouth opening phase, during which larvae sink to the bottom of rearing tanks. Upwelling systems have been used to mitigate this and they also function to keep food particles in suspension (Fielder & Heasman 2011). Not all species are as

tolerant of turbulence. For example, turbulence levels created by aeration from the centre of the larval rearing tank at rates greater than 200 $mL.min^{-1}$ resulted in significantly reduced growth and survival, and reduced rotifer predation by *L. lineata* larvae from first feeding to 14 days post hatch (dph). Combining the best rate of aeration 200 $mL.min^{-1}$ with green-water resulted in the highest growth and survival of *L. lineata* larvae (Shaw *et al.* 2010).

Green-water culture can be described as where microalgae are included within the rearing environment of larval fishes or crustaceans (Palmer *et al.* 2007). This can include pond cultures, where naturally occurring phytoplankton are encouraged to proliferate through pond fertilisation and management strategies, or more controlled tank systems where desired microalgae species are seeded or added regularly to provide favourable and continuous nutrition (Palmer *et al.* 2007). Some fish larvae may feed directly on the micro-algae, but the micro-algae are generally used to support either a natural zooplankton bloom or the green-water is also seeded with zooplankton such as rotifers, *Artemia* or copepods, or the zooplankton may be added daily, sometimes with prior enrichment (Andrade *et al.* 2010; de Melo-Costa *et al.* 2015). Higher growth and survival have been reported from green-water systems and this has been attributed to improved direct and indirect nutrition, lower stress levels, enhanced environmental conditions for feeding, improved water quality, chemical and digestive stimulants and antibacterial properties of the microalgae (Palmer *et al.* 2007). Controlled green-water culture has been used successfully for rearing larvae of a number of estuarine finfish species in Queensland Australia (Palmer *et al.* 2007).

Green-water systems, in conjunction with appropriate tank background colours, can contribute to reduced walling behaviour and jaw damage, and higher survival and growth (Cobcroft *et al.* 2012). The density of algal cells in green-water cultures can affect the success of the system with some species. For example, feeding performance of yellowtail kingfish *S. lalandi* larvae was reduced at algal cell densities higher than 16×10^4 $cells.mL^{-1}$ (Carton 2005). First-feeding *S. lalandi* larvae performed equally well in both clear-water and green-water (8×10^4 $cells.mL^{-1}$) (Carton 2005). Some aquaculture facilities continue to rear fish larvae in clear-water systems, adding enriched feeds (either live or manufactured) daily.

In this chapter we report on the effects of a range of different rearing conditions trialled on jungle perch larvae, including a range of first feeds, as well as a range of tank and pond conditions.

Objectives

The work described in this chapter relates to the general objective "Develop hatchery production techniques for jungle perch fingerlings". This is a key step toward the other general objective "Develop a jungle perch production manual for fish hatcheries".

Hatchery production begins with spawning and egg management (see Chapter 1) and is followed by larval rearing. The specific objectives in this chapter were to:

i. identify factors that influence larval survival
ii. optimise larval development through to post-metamorphosis (fry or fingerlings).

Methods

Broodstock management, spawning, egg collection and incubation

The methods for broodstock collection, broodstock management and feeding regime, spawning induction, spawning tank management, collecting eggs and evaluating fertilisation rates and egg incubation are all described in detail in Chapter 1 of this report. Management of broodstock for production of eggs and management of those eggs for use in larval rearing experiments was identical to that described in Chapter 1.

Live feed production

Five types of live feed were produced for larval rearing experiments. The copepod *Parvocalanus crassirostris* (including nauplii stages). Sydney rock oyster *Saccostrea glomerata* trochophores, super small (ss) strain rotifers *Brachionus rotundiformis*, brine shrimp *Artemia salina* and zooplankton blooms in ponds, which included various species of copepods and rotifers. The production of ss strain rotifers and copepods also required production of micro-algae. Zooplankton blooms in ponds were dependent on management of phytoplankton blooms. *Artemia salina* were used as a supplementary feed for advanced jungle perch larvae.

Production of the copepod Parvocalanus crassirostris

Seed stock of *P. crassirostris* was supplied to BIRC by the Northern Fisheries Centre in Cairns in 2012. Further seed stock of *P. crassirostris* was supplied by James Cook University, School of Marine and Tropical Biology, Aquaculture Group in 2014.

Isochrysis aff galbana (T-Iso) production

To produce copepods it is essential to supply them with the micro-alga *Isochrysis* aff *galbana* (T-Iso). Bulk production of T-Iso can be problematic in outdoor tank culture. It is feasible in cooler weather, but T-Iso cultures tend to crash in summer heat wave conditions. Unfortunately summer is the time when the largest quantities of T-Iso are required, because this coincides with the jungle perch spawning season. However, it is possible to produce reasonable quantities (up to 300 L) of T-Iso in bags under controlled temperature and light conditions indoors.

Stock cultures

Stock cultures of T-Iso were maintained axenically (i.e. without bacterial contamination) in 250 mL Erlenmeyer (conical) flasks in F medium (see below). Stock cultures were gently swirled, at least once daily and sub-cultured using axenic techniques monthly or bi-monthly.

T-Iso culture (stock and working cultures) up to 10 L were maintained in an air-conditioned room (20 to 24 °C) with fluorescent daylight spectrum lighting. From stock cultures in 250 mL Erlenmeyer flasks, working stocks were maintained in 2 L Erlenmeyer flasks and 10 L carboys, which had been prepared as described below. Bulk culture bags were seeded from one or more carboys. Aeration with CO_2 injection was provided for working cultures (2 L Erlenmeyer flasks, 10 L carboys and 300 L bags). Bulk culture bags were grown indoors, with the stock and working cultures, and outdoors, under 80% shade cloth. 200 L hard plastic translucent tubs were also used for bulk culture outdoors. Outdoor cultures generally were only consistently successful in autumn, winter and spring.

Media preparation

Approximately 10% volume of freshwater was added to clean 2 L Erlenmeyer flasks (~0.2 L) or carboys (~1.0 L) and vessels were then filled with 1 µm-filtered seawater. This ensured that any minor evaporative losses during sterilisation or culture did not result in the culture medium becoming hypersaline.

Five mL of F media nutrient solution (see below) was added to 2.0 L flasks and 10 mL of F media nutrient solution was added to 10.0 L carboys. Vessels were then capped with rubber bungs which were fitted with one open port to allow steam to escape. Airline fittings were sealed to prevent overflow, then the vessels were sterilised by autoclaving. Times and conditions for sterilisation were dependent on the model of autoclave. Once the vessels were removed from the autoclave, any temporary seals were removed and replaced with the required, sterilised fittings, and open ports were fitted with sterilised seals. Vessels were allowed to cool overnight before use.

Culture preparation

Starter cultures were harvested from flasks or carboys by siphoning. A single 2 L flask with a well-established, advanced culture was used to seed one 2 L flask and four 10 L carboys with starter cultures of 200 mL and 400 mL each, respectively. During subculturing, care was taken not to disturb the settled material at the bottom of the parent vessel. Siphon tubes were sterilised in a chlorine bath and either rinsed in freshwater or allowed to dry completely before use. Fittings on flasks and carboys were sprayed with ethanol before and after the transfer.

Bulk bags were prepared from 1850 mm x 850 mm x 100 µm plastic bags, which were sealed across the top using a Venus heat sealer. Bags were placed within a 1200 mm x 500 mm diameter cylindrical weldmesh steel frame. The top corner of the sealed bag was cut open, and bags filled with 300 L of 1 µm-filtered seawater. An air-line was then placed in the bag, with the air stone set approximately 10 cm from the bottom of the bag. The water was disinfected by the addition of 30 mL of pool chlorine (liquid sodium hypochlorite, 13% available chlorine), which was distributed by briefly aerating the bag, and then allowed to stand overnight. The following day, 2.4 g sodium thiosulphate was added to the bag to remove any residual chlorine and 11 g of the soluble fertiliser Aquasol (Yates Australia www.yates.com.au/products/fertilising/water-soluble/aquasol/) was then added. The bag was aerated to allow for mixing and dissolution of the Aquasol. A full 20 L carboy of a 1-2 week old culture of T–iso was then added, either poured directly or siphoned into the open portion of the bag. The open area of the bag was then sprayed with ethanol, and the bag sealed around the air-line with rubber bands. Bags prepared in this manner typically reached a high enough density for harvesting in approximately one week.

F medium preparation

The nutrient medium used for stock and working cultures was based on Guillard's F formula (Guillard & Ryther, 1962). The F medium was made up in concentrated form (1000 x) and added to cultures at a rate of 1 $mL.L^{-1}$.

Copepods and copepod nauplii

The production of *P. crassirostris* was based on the methods developed for copepod production at the former Northern Fisheries Centre in Cairns (Department of Primary Industries and Fisheries 2011). *P. crassirostris* are not cannibalistic, so are more easily reared than some other copepod species. They are also a relatively small species of copepod. Adults are 200 to 300 µm in length and the nauplii stages range from 40-100 µm. Copepod cultures were kept isolated from any rotifer cultures to prevent contamination. Equipment was not shared between ss strain rotifer and copepod production areas.

Copepod tank setup and maintenance

Copepod cultures were maintained in 1.1 m diameter 1000 L sky blue gel coated fibreglass tanks with a 7° conical base. To prepare for a culture the tanks were filled to a level of 300 L with 1 µm-filtered UV-treated seawater. The tank water was heated to 28 °C with a 600 W immersion heater and gently aerated through a central airstone. A scourer was hung in each tank from the centre standpipe (Figure 2.1). The scourer was used to capture flocculated algae to help maintain water quality to facilitate the harvest of copepods in screened buckets (see below). Depending on the stage of production and experimental requirements up to ten copepod tanks were run simultaneously. Limiting factors were space, capacity to produce sufficient T-Iso and labour needs.

Adult copepods were stocked into tanks at a rate of 1 $adult.mL^{-1}$ using copepods harvested from another culture tank (Day 0). When cultures were first started from seed stock the initial stocking densities were less than 1 $adult.mL^{-1}$ and only two tanks were run. The number of tanks in production was increased over several harvest cycles.

After stocking a tank with adult copepods, 15-20 L of the micro-algae (T-Iso) was added to the tank each morning. The exact volume of T-Iso required varied with the concentration of the *Isochrysis* culture. An algal cell density in the copepod tanks of 5 x 10^4 cells mL^{-1}, i.e. 50 000 cells mL^{-1} was the target density. At this density the copepod culture tank water was stained light yellow brown. The scourer was removed and washed in freshwater each day to remove accumulated flocculated algae, then placed back in the tank.

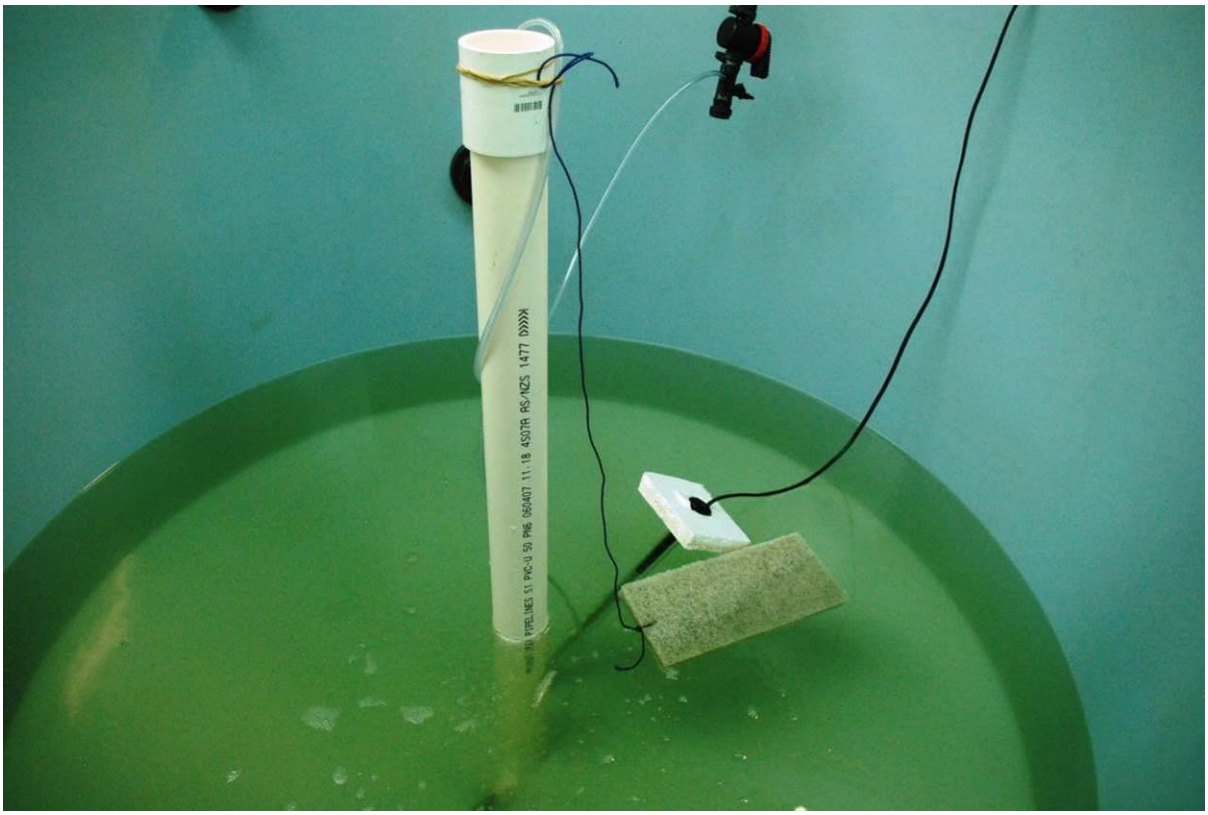

Figure 2.1: Copepod tank. Note the central stand-pipe, gentle aeration, heater, water coloured by Isochrysis and the suspended scourer.

Monitoring copepods

Nauplii normally appeared in copepod tanks one to two days after stocking adults (day 1 or 2). Nauplii take seven to nine days (days 8-11) to reach mature adult size when the tank temperature is 28 °C. By the end of the cycle the tank volume had increased to approximately 500 L with daily addition of T-Iso, and copepod densities normally rose to between 3 and 10 adults.mL^{-1}. Water temperatures were monitored daily with a thermometer to confirm conditions remained optimal. Heaters were adjusted as required. Air-stones were checked daily to ensure they were maintaining gentle aeration and mixing. If necessary, air stones were cleaned or replaced to maintain adequate levels of aeration.

Densities of copepod nauplii, copepodites and adult copepods were monitored. This was done by collecting a 100 mL water sample near to where the air supply upwelled water in the tank. Using a 2 mL pipette air was gently blown into the sample to mix it. A sub-sample was taken from the 100 mL sample with a 2 mL pipette. The contents of the pipette were viewed under a dissecting microscope and the number of adults and other stages (nauplii and copepodites) present were counted. The process was repeated for a total of 5 x 2 mL sub-samples. The mean number of adults and other stages present per 2 mL sample was calculated and converted to numbers of copepods, copepodites or nauplii per mL.

From day 9 approximately 15 mL of the 100 mL sample was placed in a Petri dish for observation under a dissecting microscope. If the majority of copepods were adults of even size and the male copepods were swimming with erratic quick movements, then the copepods were considered ready for harvesting and separating into further cultures or for stocking into jungle perch larval bowls, tanks, or ponds.

Spawning events were generally timed around predicted availability of copepods if copepod nauplii were planned to be used as a feed in an experiment. Copepod adults were stocked into jungle perch larval bowls or tanks one day after the jungle perch larvae hatched.

Harvesting copepods

Copepods were harvested by draining the copepod tank water through a screen. The screen was set into a 200 mm diameter sectioned piece of PVC pipe (Figure 2.2). The screened pipe was placed into a 20 L bucket with large drain holes cut approximately halfway up the bucket (Figure 2.2). The drain holes set the water level in the bucket to ensure copepods didn't strand on the screen and also prevent the screened pipe from overtopping. Each copepod tank was drained via a flexible outlet pipe attached to a PVC pipe end piece. The end piece had an inverted U shape and tapered to a 25 mm PVC outlet. The end piece hooks over the screened pipe (Figure 2.2).

Figure 2.2: Copepod harvesting system. From left to right: Screened PVC pipe inverted, screened pipe in harvest bucket with tank drain pipe end piece in place and harvest bucket.

Each copepod tank was drained slowly (over 15 to 20 minutes) to prevent overtopping of the screened pipe. The scourers in the tanks reduced the amount of large particles in suspension and therefore helped to reduce blocking of the screens by flocculated particles. As copepod tanks were drained the screened pipe was twisted back and forth by hand every few minutes to help prevent the screen from clogging.

Two different sized screens were used for harvesting copepods. A 124 µm screen was used to collect only adult copepods. Other stages passed through the screen. This screen was used routinely to harvest even-aged cultures to seed and build up new tank cultures. A 60 µm screen was used to capture multiple developmental stages. When a tank is harvested at 9 or 10 days after stocking it will contain mostly adult copepods, but there may be some late copepodite stages present and it is also possible that some adults may have already spawned, so some early nauplii stages may also be present. When collecting copepods to seed jungle perch larval tanks, it can be advantageous to harvest multiple stages. It will provide slightly staggered availability of nauplii in the larval tank, so can prolong the availability of nauplii stages and can help cater to larvae growing at slightly different rates.

When a copepod tank had completely drained into a screened pipe set into a bucket, the screened contents were gently flushed with 1 µm-filtered UV-treated sea water for 5 min. This was to remove any possible contamination by ciliates (that may compete for algae). If using a 124 µm screen, the flushing will also remove any ss strain rotifers should there have been any accidental contamination.

After flushing the sample, copepods were collected by submerging 250 mm plastic containers into the screened pipe in the bucket. The collected copepods were transferred to a new copepod culture tank, a jungle perch larval rearing bowl or tank or into a container of seawater for transport and release into a larval rearing pond. Copepods were released into a new tank by partially submerging the container before gently pouring out the contents to prevent any air exposure. Based on the estimated numbers of copepods from counts in pipettes (see above) harvested copepods from single tanks were split between two or more culture tanks, provided the stocking densities were somewhere between 1 and 2 adults.mL^{-1}. For jungle perch larval rearing tanks and bowls adult copepod stocking densities were generally between 1-3 adults.mL^{-1}.

Production of ss strain rotifers Brachionus rotundiformis

Nannochloropsis oculata (Nanno) production

Super small strain rotifers are reared on the micro-alga *Nannochloropsis oculata* ('Nanno'). Early production stages for Nanno, including stock cultures, media preparation and rearing in flasks and carboys were done as described for T-Iso above. However bulk production of Nanno was done outdoors in 6000 L capacity tanks. The vast majority of Nanno production was run by seeding tanks from tanks. Nanno was far more resilient to outdoor culture in summer than was T-Iso.

A Nanno culture tank was prepared by adding 4500 L of seawater through a 1 µm filter bag to a 6000 L capacity fibreglass tank. The seawater was then sterilised by addition of 450 mL of pool chlorine (application rate 100 mL per 1000 L). The tank was then left to sit overnight. The next morning the tank was dechlorinated by addition of 36 g of sodium thiosulphate (application rate 8 g per 1000 L). Following dechlorination,1000 L of Nanno was pumped from an existing culture tank to the new culture tank. The Nanno was added to the tank through a1 µm filter bag to remove any flocculated algal particles or ciliates. Secchi depth in the source tank was normally 10-15 cm. The newly seeded tank normally had a Secchi depth of approximately 35 cm. Aquasol (Yates Australia www.yates.com.au/products/fertilising/water-soluble/aquasol/) was used to fertilise the culture tank. Aquasol was applied at a rate of 35 g per 1000 L. Culture tanks were exposed to full sunlight at natural photoperiods. In summer Nanno culture tanks reached a high enough cell density (Secchi depth <15 cm) within seven days to seed other tanks or to seed green-water culture tanks for rearing fish larvae or to supply small strain rotifer rearing tanks. Culture tanks were maintained for up to 12 days from set up, while being used to feed ss strain rotifer tanks or greenwater cultures.

Super small (ss) strain rotifer rearing protocols

The ss strain rotifers were reared in 1000 L capacity fibreglass tanks, with a 7º cone base with a 50 mm drain linked to an outlet valve. Standpipes were placed in the outlet drain during culture, and a scourer was hung from the standpipe for removal of flocculating algae. The tanks were identical to those used for copepod culture. The ss strain were produced over an 8 day cycle. On day 1 tanks were filled (through a 1 µm filter bag) to 800 L with Nanno pumped from a mature Nanno culture tank. Tanks were heated to 28 ºC using 600 W immersion heaters and aerated quite vigorously via a 4 mm diameter weighted T piece at the end of a plastic airline. Tanks were seeded with ss strain rotifers at a rate of 40 mL^{-1}. The rotifers for seeding were obtained by harvesting from an existing culture tank by drain harvest.

On day 4 an additional 200 L of Nanno was supplied to the ss strain rotifer tanks using the same methods as for the supply of the initial 800 L of Nanno on day 1. On days six and seven, 25 g of yeast was added to each of the ss strain rotifer tanks as a supplementary feed. On day eight ss strain rotifers were counted and harvested.

Counting and harvesting ss strain rotifers

Counting of ss strain rotifers followed the same method as described for monitoring of copepods above, except a 1 mL pipette was used for counting of ss strain rotifers, as compared to a 2 mL pipette

for copepods. Generally by day 8, rotifer densities in the culture tank were around 200 rotifers.mL^{-1}. To achieve a density of 40 rotifers.mL^{-1} in a 1000 L tank, 200 L would need to be harvested from the 1000 L culture tank. Harvest volumes were adjusted up or down according to the density of ss strain rotifers recorded at day eight in the culture tank.

Harvest of ss strain rotifers essentially followed the methods outlined above for copepod harvest, using the same type of drainage pipe apparatus. However, the screen size used for harvesting ss strain was 44 µm. When the desired volume of tank water had been run through the screened harvester, rotifers were washed in the harvester with 1µm filtered Nanno. Rotifers were then scooped from the harvester with 250 mL plastic containers and transferred to either new culture tanks or jungle perch larvae green-water culture tanks.

Oyster trochophore production

Over 120 live Sydney rock oysters were purchased from a commercial supplier and held short term in oyster baskets suspended in a concrete raceway fed with raw seawater until required for spawning. To produce oyster trochophores, oysters were strip spawned. The advantage of strip spawning over heat induction spawning is that larvae can be produced on demand. For larval bowl rearing experiments only a small number of oysters were required to produce sufficient numbers of trochophores. Normally two to three females and one male were sufficient each day. However trochophores were supplied daily during experiments.

Oysters for stripping were removed from the oyster basket, scrubbed clean with a coarse nylon brush and rinsed in UV treated 1µm filtered seawater. Oysters were then opened one at a time with an oyster chucking knife. Gonad condition was checked and if the gonad appeared well developed a small biopsy was taken with the tip of a pipette and examined under a microscope to confirm the sex of the oyster. Gonads were stripped of gametes by scarifying with a scalpel blade and washing the gametes free with UV treated 1 µm filtered seawater from a wash bottle. The female contents were washed into a 3 L container of treated filtered seawater. Male gonads were washed into a 500 mL beaker. Female gonads were always processed first. After stripping, the female gonad contents were strained through a 100 µm sieve into another 3 L container to remove any lumps and to separate individual eggs. Eggs were then gently washed with 1 µm filtered UV treated seawater on a 15 µm screen set in a 200 mm PVC pipe before backwashing into a 3 L container.

Washed eggs were then observed under a light microscope (100 x magnification) to confirm water hardening. A 0.1 mL sub-sample of eggs was taken by pipette and placed in a small glass well and viewed under a dissecting microscope to obtain a count of eggs. If eggs were too numerous to count accurately, the pipette sub-sample was diluted by a factor of 10 and a 0.1 mL sample taken from the diluted sub-sample. The dilution factor was taken into account for any calculations of densities of eggs in the bowl. This process was repeated five times to determine an average density of eggs. The total number of eggs in the container was then determined volumetrically. Next a sample of sperm was checked under a light microscope at 200 x magnification to check for sperm motility. If sperm was motile it was used to fertilise the eggs. If sperm was of poor quality another male oyster would be stripped.

Sperm was added to the container of eggs with a 2 mL pipette. After adding sperm, a subsample of eggs was viewed under the light microscope. If around five sperm could be seen around each egg then it was considered sufficient sperm had been added to the container of eggs. If enough sperm were not seen then further sperm was added to the bowl incrementally until sufficient sperm were seen.

From 20 minutes after adding sperm, eggs were examined for polar body formation. If the majority of eggs were observed to have polar bodies form, then it was considered that sufficient fertilisation had been achieved. Fertilised eggs were then added to larval rearing bowls (or tanks) to achieve a density of 50 eggs mL^{-1}. Eggs would hatch in the larval rearing bowls or tanks. The volume of eggs required to achieve the desired density in the larval bowls was calculated based on the known density of eggs in the spawning container. Given the high density of eggs in the spawning container, this generally

required adding a volume of less than 2 mL of eggs to a larval rearing bowl to achieve the desired density.

Pond plankton production

The objective of pond production was to promote and sustain a zooplankton bloom as long as possible for rearing of fish larvae. Ponds were dried out before preparation. Three to four weeks prior to a proposed spawning, pond preparation began. Dolomite or lime was spread over the dry pond bed. Dolomite or lime application rates for different sized ponds used for larval rearing experiments are shown in Table 2.1.

Table 2.1: Dolomite or lime application rates for different sized ponds

Pond size **Pond Volume**	15 m x 15 m 400 000 L	40 m x 40 m 3.2 million L	100 m x 50 m 10 million L
Lime or dolomite application rate	5 kg	40 kg	120 kg

A mixture of inorganic and organic fertilisers was then added to the pond. For the initial fertilisation of a pond, fertiliser was added either to the dry pond bed or as the pond filled. The quantity of inorganic and organic fertilisers used varied according to pond size. Application rates are shown in Table 2.2.

All ponds were filled with raw seawater because the objective was to introduce copepods, other zooplankton and phytoplankton. A 300-400 μm screen was fitted as a sock to the pond inlet pipe to prevent wild fish eggs or fish larvae or other fauna that may compete with or prey on jungle perch larvae. However, this screen still allowed copepods other small zooplankton and phytoplankton to enter the pond.

When the pond was full, a paddle wheel or vigorous aeration was used to help mix pond water and distribute fertiliser throughout the pond. However, paddlewheels were not used once jungle perch embryos or larvae were stocked in the pond. After the initial fertilisation of the pond, follow up fertilising (Table 2.2) was applied twice per week to sustain phytoplankton blooms and the copepods and other zooplankton that feed on the phytoplankton.

Table 2.2: Pond fertilisation rates

	Pond 15 m x 15 m 400 000 L		**Pond 40 m x 40 m 3.2million L**		**½ ha pond 50 m x 100 m 10million L**	
Fertilizer	**Initial**	**Follow up twice weekly**	**Initial**	**Follow up twice weekly**	**Initial**	**Follow up twice weekly**
Monoammonium phosphate (kg)	0.143	0.024	1.14	0.19	3.563	0.594
Urea (kg)	0.712	0.119	5.7	0.95	17.813	2.968
Potassium nitrate (kg)	0.645	0.107	5.26	0.876	16.438	2.688
Pollard or bran (kg)	1.25	0.25	10	2	31.25	6.25
Lucerne chaff (kg)	1.25	0.25	10	2	31.25	6.25

Seeding the pond with copepods

Copepods normally entered the ponds in raw seawater pumped in from the ocean or estuary (Pumicestone Passage). The species composition and abundance of copepods pumped into the pond varied according to prevailing conditions in the source water. To create more certainty in development of a copepod bloom some 400 000 L ponds were seeded with cultivated copepods or with copepods captured by plankton net from successfully blooming ponds. Within a week of the initial fertilisation of a 400 000 L pond a phytoplankton bloom was usually apparent. At this stage two 600 L tanks of copepods *P. crassirostris* were harvested (approximately 3 million copepods) from copepod culture tanks and added to the pond. Follow up stockings of similar number of copepods were made nine days later and again when jungle perch larvae were released into the pond. The stocked copepods built up numbers naturally in the pond and supplemented the natural multi-species copepod and other zooplankton blooms from the raw seawater.

Seeding of copepods from pond to pond used a copepod bloom in a 3.2 million L prawn pond containing prawns already weaned onto commercial pellet feeds. Copepods were captured from the prawn pond with a plankton-net without impacting on the pond prawn production. Copepods captured with a plankton-net were transferred to a bucket containing some pond water. Care was taken to empty the plankton net into the bucket keeping the collecting end of the net submerged in the water. It was important not to expose copepods to air as this can prevent moulting and development of immature stages. The open mouth of the plankton net had a surface area of 1000 cm^2. Five x 40 m sweeps were run through the prawn pond to collect plankton for seeding of a 400 000 L pond. This equated to sampling 20 000 L of water for zooplankton.

Monitoring the pond plankton bloom

Monitoring the pond plankton blooms was relatively simple. In the weeks prior to stocking the pond with jungle perch larvae, a 2 L water sample was collected from the pond two to three times per week in a plastic Nalgene bottle. If the pond was aerated the water sample was collected near the aeration point because the water was well mixed. To collect pond water the 2 L Nalgene bottle was submerged 50 cm below the surface. The sample was then concentrated in the laboratory through a 20 μm filter to a volume of 200 mL. A pipette was used to take six 1 mL sub-samples from the concentrated sample. Gentle blowing down the pipette was used to mix the concentrated sample before taking each subsample.

The subsamples were placed into wells for viewing under a dissecting microscope. The number of copepod nauplii, copepodites and adult copepods from the different key copepod families (Harpacticoid, Calanoid and Cyclopoid) were counted, along with other zooplankton groups including rotifers, ciliated protozoans, mollusc trochophores, barnacle nauplii and polychaete larvae. From the six sub-samples the average number of each zooplankton group per mL of subsample was calculated. The average count per concentrated subsample was multiplied by 100 to estimate the total count per litre of pond water.

Plankton monitoring was continued at regular intervals after the stocking of jungle perch larvae in ponds. This is described further under pond rearing experiments.

Managing the pond plankton bloom and filamentous algae

To maintain the plankton bloom in the pond, follow up fertilising of the pond was completed twice weekly (Table 2.2). Use of lucerne chaff and pollard was stopped between days four and eight after hatch. After day eight these organic fertilisers were reinstated to the fertilising protocol. Between days four and eight jungle perch larvae were inflating their swim bladders. Pollard and lucerne can promote scum formation on the surface that could potentially inhibit swim bladder inflation.

Regular application of fertiliser can promote growth of filamentous algae and *Ulva* (sea lettuce). These algae can compete with phytoplankton for nutrients, and if they proliferate on the bottom of the pond,

can create problems for harvesting of fingerlings. If pond Secchi depths began to exceed 1 metre, then a non-toxic blue dye (Alpine Blue or equivalent product) was added to the pond to help shade the pond bottom and prevent filamentous algal growth. The blue dye was applied to the pond following the manufacturer's recommended rates. With aeration, the pond water turned over regularly and phytoplankton were still able to be exposed to the light in the surface layers and could therefore continue to bloom. The blue dye was therefore used to help prolong phytoplankton blooms and zooplankton blooms by reducing competition from filamentous algae. For ponds not covered with bird netting, blue dye can also afford some protection to fingerlings from predatory birds.

Hydras can also be a problem for pond rearing of larvae. Hydras form branching anemone-like colonies. Some hydras can be harmful to early stage larvae, but most hydras prey on copepods, and therefore impact on the larval food supply. Therefore, as far as is possible, any hydras spotted in the pond were removed. Those growing on the edge near the surface were removed by hand. Larger hydras attached to the bottom were scooped out with a coarse meshed long handled dip net.

Further details on pond management relating to larval rearing are described under pond rearing experiments below.

Brine shrimp Artemia salina production

Artemia salina (commonly known as brine shrimp or Artemia) nauplii can be used as a feed for more advanced jungle perch larvae. Production of Artemia was quite straightforward. We used Artemia that are supplied as pre-treated cysts impregnated with magnetic material (Sep-Art). Use of a magnetic separator (Sep-Art separator) enables harvest of completely cyst-free Artemia nauplii.

The Artemia production process used at BIRC was as follows. A 500 L egg cup tank was filled with 1 μm filtered, UV treated seawater and heated to 27-28 °C. The tank was vigorously aerated and then a 425 g container of Sep-Art Artemia cysts were added to the tank. The tank was left for 24 hours for the cysts to hatch. Hatching was confirmed prior to harvesting by examining a 10 mL sample of tank water under a dissecting microscope. After hatching was confirmed the tank aeration was shut off and the tank water allowed to settle. A lid was then placed over the tank to block off any light in order to reduce the number of Artemia nauplii near the surface.

The bottom outlet of the tank was connected to an outlet drain pipe which was directed to a cylinder containing magnets (Sep-Art separator) which overflowed into a 125 μm mesh bag tied onto the end of the cylinder and set into a bucket of seawater. The valve on the outlet of the egg-cup tank was opened to allow a flow of approximately 20 $L.min^{-1}$ through the Sep-Art separator into the harvest bag. Artemia nauplii collected in the bag and any cysts were removed by the magnet system. After the tank was completely drained, the bag was flushed with a flow of 1 μm filtered, UV treated seawater. Next the bag was turned inside out into a half full 20 L bucket of filtered UV treated seawater and washed down with a flow of the same water to ensure all Artemia nauplii were flushed into the bucket.

One 425 g container of Artemia cysts was sufficient to stock two 15 m x 15 m x 1.8 m (depth) ponds for two days. Approximately 250 000 nauplii were produced per gram of cysts. Artemia feeding was only required for a week to 10 days while jungle perch larvae were weaned onto powdered feeds, and was only required when pond copepod blooms were waning (see ponds for rearing experiments below).

Larval bowl experiments

Larval bowls (Figure 2.3) with a capacity of 3 L were used to increase replication in some multifactorial experiments. This level of replication would not have been achievable in terms of space or food supply if conducted in standard larval rearing tanks. Copepod availability in particular was a constraining factor for the number of tank replicates that could be used. The experimental designs for the bowl based experiments are summarised in Table 2.3. One experiment that compared diets was not multifactorial.

Figure 2.3: A 3 L larval rearing bowl set in a heated water bath. Note the heater in the background (top right) and label on the bowl.

Larval bowls were set in heated water baths (troughs). Two 500 W submersible heaters were used in each trough to regulate water temperatures. With the exception of the background colour experiment, where multiple background colours were used, larval bowls were covered on the outside with a light blue plastic sheet (held on by elastic bands) to provide a light blue background. All bowls were labelled to indicate the experimental treatment and replicate number.

All treatments were randomised, although temperature treatments were blocked by water bath (troughs). However, more than one water bath was used for each temperature block and the order of blocks was randomised. Each trough (water bath) could hold six larval bowls. Figure 2.4 shows larval bowls used in the background colour x lighting intensity x broodstock diet experiment.

Table 2.3: Summary of larval bowl multifactorial experimental designs.

Multifactorial experiment	Treatment descriptions	Number of replicates	Comments
Temperature x larval diet	Temperature: 25°, 28°, and 31 °C Larval diet: ss strain rotifer and oyster trochophore	Temperature (3) x larval diet (2) x 6 replicates = 36 bowls 12 bowls for each temperature Two troughs (water baths) for each temperature. 18 bowls for each larval diet	Temperature treatments blocked by water bath, but two blocks for each temperature treatment and position of blocks randomised. Other components randomised within blocks. Overall design balanced and randomised. Starting salinity set at 35 ppt. Light ambient indoor approx. 400 lux. 10 hours dark, 14 hours light. Broodstock diet standard 400 embryos per bowl.
Background colour x lighting intensity x broodstock diet treatment	Background colour: blue, yellow, white, black Light intensity: Dull (4-8 lux) Bright (380-950 lux) Broodstock diet: standard and terrestrial invertebrate	Background (4) x Light intensity (2) x Broodstock diet (2) x 3 replicates = 48 bowls 12 bowls for each colour 24 bowls for each light intensity 24 bowls for each broodstock diet group	Larvae fed on copepod nauplii. Temperature set at 28 °C and starting salinity at 35 ppt. 10 hours dark, 14 hours light Illumination by fluorescent lighting suspended above troughs. Reduced lighting provided by screening with shade cloth. 250 embryos per bowl. Repeated with 200 embryos per bowl
Larval diet	3 x diets ss strain rotifer, copepod *P. Crassirostris* nauplii and oyster trochophores	Diets (3) x 8 replicates = 24 bowls	Temperature set at 28 °C and starting salinity 35 ppt 10 hours dark 14 hours light Pale blue background colour Illumination approx. 400 lux Broodstock diet standard Position of diet treatments randomised within troughs. Four troughs, six bowls per trough 200 embryos per bowl

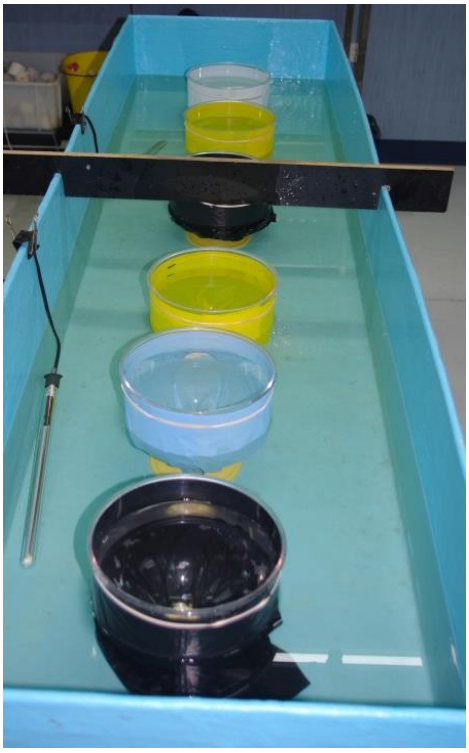

Figure 2.4: Coloured bowls arranged randomly in a trough used as a heated water bath.

All bowls were filled to 2.2 L volume with 1 µm filtered UV treated seawater. Those used in the temperature x larval diet trial were chlorinated with 0.07 mL of chlorine that had been diluted by adding 1 mL of chlorine to 100 mL of filtered seawater. Bowls were left to stand overnight then dechlorinated with 0.0036 g sodium thiosulphate (anhydrous). Approximately 400 late stage jungle perch embryos were added to each bowl for this experiment. Numbers were estimated volumetrically. For example, if the density of skimmed embryos (see Chapter 1) in a container was 50 mL^{-1}, then 8 mL of embryos would be added to a bowl one day post hatch 240 mL of 1 µm filtered Nanno and 120 mL of T-Iso (from bag cultures) was added to each bowl. Bowls stocked with ss strain rotifers were stocked at a rate of 20 rotifers.mL^{-1}. Bowls stocked with trochophores were stocked at a rate of 50 trochophores.mL^{-1} from two days posthatch and daily thereafter as required. Rotifers were not stocked daily as they were able to reproduce in the bowls, but numbers were monitored so that they could be topped up if necessary. If algae were visible in a bowl no further algae were added, but if bowls were becoming clear a further 80 mL of Nanno and 40 mL of T-Iso were added to the bowls from three days post-hatch as required.

In the case of the background colour x light intensity x broodstock diet treatment trial and for the larval diet experiment bowls were filled with 1 µm filtered UV treated seawater to a volume of 2.45 L. This water was not chlorinated then dechlorinated, because copepods are sensitive to low levels of chlorine and sodium thiosulphate. The bowls for the larval diet experiment were stocked with late stage jungle perch embryos at a rate of 200 per bowl. The background colour multifactorial trial was run twice, once with a density of 250 larvae per bowl (stocked as late stage embryos) and once with 200 larvae per bowl. For the trial with multiple background colours, 50% of bowls received embryos derived from broodstock on the standard diet and 50% received embryos derived from broodstock on the terrestrial invertebrate diet. In the larval diet experiment all larvae were produced from broodstock on the standard diet treatment.

From one day post hatch 50 mL of T-Iso (from carboys) was added to each bowl. From two days post hatch adult copepods were stocked in each bowl at a rate of 3 adults.mL^{-1}. T-Iso was added daily at a rate of 50 mL per bowl. Copepod nauplii densities were monitored in bowls. Four days post-hatch

further adult copepods were added to larval rearing bowls at a rate of between 1 and 2 copepod adults.mL^{-1}.

For those bowls in the larval diet experiment receiving ss strain rotifers, ss rotifers were stocked into bowls at two days post hatch at a rate of 20 rotifers.mL^{-1} and oyster trochophores were stocked daily from two days post hatch at a rate of 30 trochophores.mL^{-1}.

In all experiments larval bowls were not aerated. Jungle perch larvae are particularly sensitive to physical damage and it was not feasible to aerate such small volume bowls at a low enough level to prevent physical buffeting. It was considered the surface area to volume ratio was sufficient to provide adequate oxygen. Maintaining water quality in static systems was of importance. As required, the surface of bowls were cleaned of surface scum by drawing a cloth wipe gently across the surface. Dead larvae were siphoned from the bottom of the bowls with 4 mm silica tubes attached to a fine glass tipped tube. Water was siphoned into a bucket such that any live larvae accidentally removed could be returned to a bowl. Each day 50 mL of bowl water was removed by siphoning through 40 µm mesh, then replaced with an equivalent volume of 1 µm filtered UV treated seawater. Any evaporative losses were replaced with reverse osmosis treated freshwater.

Larvae numbers were estimated volumetrically each day in all bowls for both experiments. Three 50 mL subsamples were removed with a small plastic container each day from each bowl. The number of live larvae in each container was counted, then larvae were returned to the bowl by gently empting the container (partially submerged) back into the bowl. Containers were washed in freshwater between uses in each bowl. The mean number of larvae per 50 mL sample from each bowl was then used to estimate the number of larvae in the bowl. If no larvae were captured in 50 mL subsamples, the contents of the entire bowl were observed very carefully and a total count of live larvae was made. The experiments were intended to run for up to eight days but were terminated early if no live larvae were observed in any bowls. In the larval diet experiment larval counts commenced three days post-hatch, when exogenous feeding commences. In all other experiments larval counts commenced from day of hatch.

General observations were made of larval activity. The classic S strike posture was looked for as evidence of feeding or attempted feeding.

Tank based experiments

General tank set ups

Two types of tanks were used for most larval rearing experiments. Sky blue coloured gel coated fibreglass small tanks (capacity 1000 L, diameter 1.1 m) or large tank (capacity 7000 L (diameter 3.1 m). For some opportunistic larval rearing experiments 7000 L sky blue coloured gel coated fibreglass small tanks were used. All tanks had a 7° cone base with a central 50 mm drainage outlet. Drains were fitted with a central standpipe and all tanks were supplied with immersion heaters. Small tanks were fitted with 600 W immersion heaters (Figure 2.5) and large tanks were fitted with 3000 W immersion heaters. Tanks supplied with rotifers or trochophores as larval feed underwent a chlorination and dechlorination process as outlined in Palmer *et al.* (2007). Tanks supplied with copepods as larval feed were filled with 1 µm filtered UV treated seawater without chlorination. Standpipes fitted in tanks that were supplied with copepods as a larval feed, had drainage holes set part way up to fix the water depth and to permit water exchange or drainage. In these cases stand pipes were surrounded by a 60 µm screen to prevent loss of copepod nauplii (Figure 2.5). The screens on the large tank stand pipes used for an opportunistic larval rearing trial were 125 µm. They also facilitated water exchange and retained jungle perch larvae, but did not retain copepod nauplii.

None of the larval rearing tanks were filled to capacity (see individual experiments below). Small tanks were fitted with one surface skimmer and large tanks were fitted with two surface skimmers to remove lipid and protein scum from the surface. Surface skimmers were made of floating styrofoam blocks with a tear-drop shaped opening cut into it (Figure 2.5). A jet of air was directed into the

narrow tear-drop opening via a bent glass tube to force surface scum inside. Scum was blotted from the surface of the tear drop area twice daily with a cloth wipe. Tank experiments using copepods were also supplied with the microbial water conditioner Sanolife MIC-F, containing *Bacillus subtilus*, *Bacillus lichenformis* and *Bacillus pumilus.* The microbial water conditioner was applied at the rates and intervals recommended by the manufacturer.

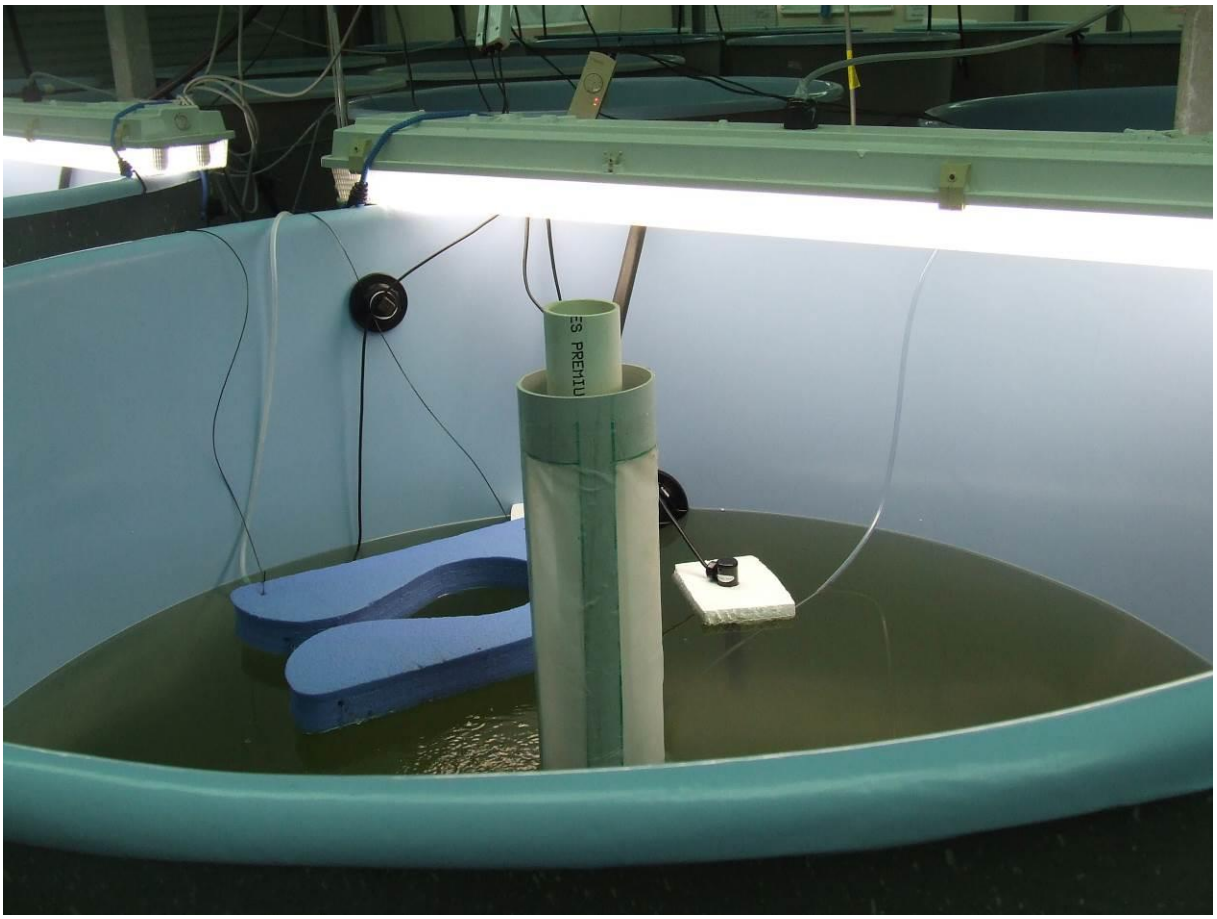

Figure 2.5: Illuminated 1000 L capacity larval rearing tank. Note screened central standpipe, surface skimmer and immersion heater. The water contains the micro-alga T-Iso.

Light and salinity

A trial was run to examine the effects of light and salinity on larval survival. Oyster trochophores were used as the larval feed, given past failures with ss strain rotifers and lack of sufficient quantities of copepods for a large scale tank trial. Eighteen tanks were used in the trial. Nine tanks were filled to 500 L with 1 µm filtered UV treated seawater (salinity 35 ppt) and nine tanks were filled with 400 L of 1 µm filtered, UV treated seawater, then diluted by addition of 100 L of 1 µm filtered dechlorinated freshwater for a salinity of 28 ppt. The tanks for each salinity level were selected at random.

Nine tanks were covered with 70% shade cloth and six of the nine (randomly selected) were covered with a double layer of shade cloth. The position of all shade cloth covered tanks was randomised. All tanks in the larval rearing room were exposed to ambient daylight through shade cloth covered skylights. All tanks were exposed to a natural photoperiod (approximately 11 hours dark, 13 hours light). The position of the tanks in the room and the position of shade cloth-covered tanks created a variety of lighting conditions, ranging from 4 to 750 lux. The tank salinities and light conditions (as measured at the water surface) are summarised in Table 2.4.

Table 2.4: Salinity and water surface light conditions at midday, in tanks used for the light and salinity trial

Tank number	Salinity ppt	Light level lux
1	35	750
2	35	750
3	28	670
4	35	680
5	28	21
6	28	810
7	28	90
8	35	120
9	28	9
10	35	130
11	35	4
12	35	5
13	28	190
14	35	190
15	28	6
16	35	18
17	28	540
18	28	690

These tanks were run as clear water cultures. Tanks were gently aerated via a central air-stone and heated to 28 °C. Trochophores were added daily to each tank (from two days post jungle perch larval hatch) at a rate of 30 mL^{-1}. Larvae were stocked into tanks as late stage embryos. Each tank was stocked with 6400 embryos.

Jungle perch larvae numbers were estimated daily by taking 3 x 2 L sub-samples from each tank. Numbers of live larvae in each sub-sample were counted to provide an estimate of the mean number of larvae L^{-1}. This number was then used to estimate the total number of larvae remaining in a tank. After counting, larvae were gently returned to the tank. If no larvae were found in any subsamples from a tank, direct observations of larvae were made in the tank and a count of the total number of live larvae seen was made. General observations of larval behaviour were also recorded.

Opportunistic copepod feeding trials and transitioning from copepods to ss strain rotifers

Small tank trial

In January 2013 BIRC received seed stock of the copepod *P. crassirostris*. By February 2013 there were enough copepods available for an opportunistic trial of copepods as a larval feed in a 1000 L tank. However, there were insufficient copepods for replication so this trial was run as an information gathering exercise. The tank was filled to 600 L and stocked with 20 jungle perch embryos.mL^{-1}. The tank was stocked with copepods at a rate of 4 adults.mL^{-1} on day 1. The tank was moderately aerated and the temperature was set at 28 °C. The tank was held at ambient light under a double shade cloth-covered skylight at natural photoperiod (approx. 11 h dark 13 h light). In the middle of the day light levels at the surface of the tank were approximately 190 lux. This was not a formal experiment, but larvae were observed daily for signs of feeding behaviour and densities were sampled by taking 3 x 2 L samples of tank water daily with a plastic jug and counting the number of live larvae per sub-sample. Larvae were returned to the tank after counting. Bag cultured T-Iso was added to the tank at a rate of 20 L per day.

Large tank trials

Further opportunistic trials were run in March and April 2013. These trials took advantage of the availability of copepods and surplus jungle perch eggs and larvae for other experimental requirements. In these trials, large tanks were filled to a volume of 6000 L. Tanks were gently aerated via an air-stone set near the centre of each tank. There were only sufficient copepods available to stock a single tank in each trial. Therefore there was no replication. These trials were treated as information gathering exercises to help improve other tank based experiments involving use of copepods. These tanks were situated under overhead (approximately 1.5 m above the water surface) fluorescent lighting and located near a roller door that remained open during daylight hours. Light levels at the surface of the tank in March ranged between 250 and 550 lux during daylight hours. Photoperiod was 13 h light, 11 h dark. In April lighting was supplemented by placing a bank of fluorescent lights over one side of the tank approximately 50 cm above the water surface (Figure 2.6). This increased light levels on one side of the tank to 1500-2000 lux. A timer was used to run the light bank for a 14 h light 10 h dark cycle.

In March 2013, 31 000 late stage embryos were stocked into the tank, whereas in April 21 000 late stage embryos were stocked into the tank. Both trials maintained tank temperature at 28 ± 0.5 °C. Copepods were stocked at 2 copepods.mL^{-1} one day post hatch of larvae in both the March and April trials. In the March trial ss strain rotifers were stocked in the tank three days post hatch at a density of 1 rotifer.mL^{-1} to supplement copepod numbers. In March T-Iso was added to the tank daily at a rate of 20 L per day from a bag culture, plus half a carboy per day of high density T-Iso. The March trial was terminated eight days post hatch of jungle perch larvae.

In the April trial T-Iso was sourced from bag cultures and added to the tank at a rate of between 30 L and 90 L per day. The amount added was varied according to the density of the available culture. Additional adult copepods were added to the tank three days post hatch at a rate of 1 copepod.mL^{-1}. Six days post hatch ss strain rotifers were added at a rate of 1 rotifer.mL^{-1} as a supplementary feed to copepods. Further copepods were added 10 days post hatch at a rate of 1 copepod.mL^{-1}. Thirteen days post hatch ss strain rotifers were added again at a rate of 4 rotifers.mL^{-1}.

To maintain water quality it was considered essential to do a tank water change. Overnight between days thirteen and fourteen post hatch, a timer tap was used to exchange 100% of the tank water volume overnight with UV treated, 1 µm filtered seawater. The water change was expected to remove many copepod nauplii. On the morning of day 14 post hatch ss strain rotifers enriched overnight on a diet of T-Iso were added to the tank at a rate of 6 rotifers.mL^{-1}. The daily addition of T-Iso continued. The April trial was terminated 15 days post hatch.

In both the March and April trials, larval densities were estimated daily by taking five 2 L subsamples with a jug. Numbers of live larvae present in the jugs were counted to calculate a mean density per L, and to obtain an overall estimate of larval numbers in the tanks. After counting a sample the jug was emptied gently back into the tank to return the live larvae to the tank. Two to three larvae were also collected each day for observation of development under a microscope and for measurement with the QCapture pro 5.1® measuring tool. Copepod nauplii, copepodite and adult copepod densities were monitored daily. A 50 mL subsample of tank water was collected from near the centre of the tank above the aeration point. From this sample six 1 mL samples were collected by pipette, placed into viewing wells and examined under a stereo dissecting microscope to obtain counts. If densities were low, 5 mL samples were observed instead of 1 mL samples. Counts of ss strain rotifers were also made after they had been added to the tank.

Preliminary swim-bladder inflation experiment

For this experiment a 7000 L tank was half-filled (3500 L) with UV treated 1 µm filtered seawater. A bank of four daylight spectrum fluorescent lights lit one side of the tank to 1500 lux. A similar arrangement is shown in Figure 2.6. The ambient room lighting on the unlit side of the tank was 100 lux. A timer was used to give the tank a 16 h light and 8 h dark lighting regime. The tank was lightly aerated near the centre standpipe and stocked with day-of-hatch larvae at a density of 20 larvae.L^{-1}. Two protein skimmers were run in the tank to keep the surface clear of lipid and protein scum. *Isochrysis* (T-Iso) was added to the tank from tub or bag cultures at a rate of 70 L per day. Adult copepods (harvested using the 60 µm filter) were stocked into the tank one day post hatch at a rate of approximately 2 copepods.mL^{-1}. This achieved a density of approximately 5 nauplii.mL^{-1}. The experiment was not replicated due to insufficient copepod supply. Each day five 2 L samples were taken from the tank with a plastic beaker at evenly spaced intervals around the tank, approximately 30 cm from the edge. The number of live larvae in each 2 L sub-sample was counted. This provided estimates of larval densities and survival. After counting, the majority of larvae were returned unharmed to the tank. From two days post hatch 20 larvae were collected each day for viewing with an inverted light microscope (Nikon Eclipse Ti) at either 20 x or 40 x magnification, depending on the size of the larvae. Larvae were viewed in a lateral position and were examined for the presence or absence of an inflated swim-bladder. The number of larvae with or without inflated swim-bladders was recorded. Digital images of larvae were captured using a microscope mounted Nikon DS-Fi2 camera and saved using NIS Elements 4.0 software. A measurement tool in NIS elements 4.0 was used to calculate the total length of each larva examined. The experiment was run until seven days post hatch.

Figure 2.6: Fluorescent light bank set on one side of a 7000 L tank.

Swim-bladder inflation and larval survival in large tanks and small tanks

Swim-bladder inflation was compared between larvae reared in two large tanks (3.1 m diameter), filled to 2500 L and larvae reared in two smaller tanks (1.1 m diameter), filled to 600 L. All tanks were filled with UV treated 1 µm filtered seawater. Tanks were lightly aerated. Large tanks used two surface skimmers each, small tanks were fitted with one each. Tanks were stocked with late stage embryos at a density of 22.5 embryos.L^{-1}. Copepods were stocked one day post hatch. *Isochrysis* (T-Iso) was added daily to the tanks at a rate of 50 L per day to the large tanks and 12 L per day to the small tanks. Nauplii densities achieved were <1 nauplius.mL^{-1}. Tanks were lit on one side by fluorescent lights as for the preliminary swim bladder inflation experiment. Large tanks were lit by four lights and small tanks by one light. Larvae were exposed to a 16 h light, 8 h dark cycle.

From two days post hatch, 20 larvae per tank per day were examined for swim-bladder inflation as described above. As larval densities declined late in the experiment a minimum of 10 larvae were collected from some tanks. Larval survival was determined by taking five 2 L subsamples of larvae daily from each tank for counting of live larvae. This enabled calculation of mean densities per L and therefore an estimate of survival. After counting, larvae were gently returned to their respective tanks. General behavioural observations of larvae in the tanks were also recorded.

Lighting levels

Four 1000 L tanks were used to compare survival and swim bladder inflation of jungle perch larvae under lit (2500 lux) and ambient (150 lux) lighting conditions. Two tanks were exposed to ambient lighting through overhead shade cloth covered skylights and two tanks were lit by daylight spectrum fluorescent lights set 50 cm above the water surface (see Figure 2.5). Ambient lighting tanks also received some light from spillage from the directly illuminated tanks and limited fluorescent roof lighting in the hatchery that was not directly above the ambient lighting treatment tanks. Photoperiod was set at 16 h light and 8 h dark.

Tanks were filled to 600 L with UV treated 1 µm filtered seawater and heated to 28 ºC. Tanks were conditioned with a microbial water conditioner. Screened drain holes in the central standpipe fixed the water level at 600 L (see general tank set ups). Jungle perch larvae were stocked into tanks at the late embryo stage at a density of 20 embryos.L^{-1}. All tanks were seeded with copepods one day post hatch of larvae. Copepods were harvested using the 60 µm screen method (see "Harvesting Copepods" above). Adult copepods were stocked at a density of approximately 1 copepod.mL^{-1} in all tanks. Between 15 L and 40 L of T-Iso was added to each tank each day, depending on the density of the available T-Iso bag culture. This also provided some water exchange (2.5-6%) as tanks overflowed through the screened standpipe. On day six post hatch tanks were given a 50% water change by trickling in UV treated 1 µm filtered seawater. After flushing, additional 60 µm filter harvested copepods were added to the tanks at a rate of 0.5 adults.mL^{-1}.

From one day post hatch densities of larvae in the tanks were estimated by taking five 2 L sub-samples from each tank with a plastic jug. The number of live larvae in each sub-sample was counted in order to provide an estimate of mean larval density per L. This provided a volumetric estimate of abundance of larvae (and therefore survival of larvae) in each tank. After counting, larvae were returned to their tanks unharmed. From two days post hatch a sub-sample of 10 larvae per tank were taken for viewing under an inverted light microscope for the presence or absence of an inflated swim bladder. The number of larvae with or without inflated swim-bladders was recorded. The microscope, camera and viewing techniques were the same as described in the preliminary swim-bladder inflation experiment. A measurement tool in NIS Elements 4.0 was used to calculate the total length of each larva examined. The experiment was run until eight days post-hatch, after which remaining larvae were stocked into a nursery pond.

Larval survival in gentle aeration and upwelling systems

Survival was compared between larvae reared in lightly aerated tanks and tanks fitted with an upwelling system. Larvae were reared in small (1000 L capacity) tanks filled to 600 L with 1 µm filtered UV treated seawater. Two tanks were used for each treatment. Lightly aerated tanks had an air-stone set at the base of the central standpipe, and air was allowed to bubble at a gentle rate to provide oxygenation with minimal turbulence. The upwelling system used pre-aerated UV treated, 1 µm filtered seawater overflowed from an adjacent tank up through the central drain (where the standpipe would normally sit) at a rate of 2 L min^{-1}. Overflow was through an outlet set at the 600 L level in the side of the tank. The outlet was screened with 60 µm mesh fixed to a horizontal 50 mm PVC pipe, to prevent loss of copepods and nauplii from the system. Larvae were stocked as late stage embryos at a rate of 22.5 L^{-1} into all tanks. Fluorescent daylight spectrum lighting was mounted on one side of each tank providing illumination of approximately 2500 lux. Tanks were heated to 28 ºC. Water in the source tank for the upwelling system was also heated. One day after hatch, copepods were stocked into all tanks at the rate of one adult mL^{-1}. Copepod nauplii densities were monitored and they remained similar between all tanks (just under one nauplius.mL^{-1}). T-Iso was added to all tanks daily at a rate of 12 L per day. Additional T-Iso was added to the source tank for the upwelling systems to maintain T-Iso densities at the same density despite loss through overflow. Jungle perch larval densities were monitored in tanks daily by taking five 2 L sub-samples from each tank following the same method as outlined for the other tank based experiments above. General behavioural observations of larvae were also recorded.

Blue background and granite background

Survival of jungle perch larvae in tanks with light blue backgrounds and granite backgrounds was compared. If granite backgrounds reduced jaw damage then that may increase overall survival of jungle perch larvae. Two tanks were used for each background colour treatment. Two standard sky blue 1000 L capacity tanks and two 1000 L capacity tanks lined with vinyl around the side with a granite pattern (Figure 2.7). The vinyl layers had been soaked in a separate tank for a week to leach any volatile compounds that may have been present. Tanks were set up as for most experiments. A central standpipe with external 60 µm screen was used in each tank, with gentle aeration provided by an air stone set near the base of the central standpipe. All tanks were filled to 600 L with 1 µm filtered

UV treated seawater. Microbial water conditioner was applied to each tank. Jungle perch larvae were stocked as late stage embryos at a rate of 20 embryos.L^{-1}. T-Iso was added daily to each tank at a rate of 15 to 20 L per day, depending on the density of the bag culture from which it was sourced. One day post larval hatch, copepods harvested with a 60 µm screen were stocked into each tank. Adult copepod densities were approximately 2 copepods.mL^{-1}. Further copepods (approx. 0.5 adults.mL^{-1}) were added to the tanks three days post larval hatch. Copepod and nauplii densities were monitored as outlined for other experiments above. Jungle perch larval densities were also monitored daily using five 2 L sub-samples from each tank as outlined for other experiments above.

General observations of larval behaviour were made in each tank and some larvae were taken for observations and photographing with the aid of an inverted light microscope (Nikon Eclipse Ti) at either 20 x or 40 x magnification. Digital images of larvae were captured using a microscope mounted Nikon DS-Fi2 camera and saved using NIS Elements 4.0 software.

Figure 2.7: Granite patterned vinyl lining set in a larval rearing tank.

Pond rearing trials

Over the three spawning seasons jungle perch larvae were stocked into 19 ponds of varying surface area. Some of the smaller 225 m^2 ponds were covered with clear plastic glass house domes to aid heating. However, we generally opened up the side doors of these ponds because increasing temperature was not necessary in summer, but the covers were used as an attempt to reduce dilution of pond salinity by heavy rainfall events. Numbers of larvae stocked (as a mixture of late stage embryos and yolk-sac larvae) varied according to availability. It was normally larvae surplus to tank and bowl based experimental requirements that were stocked into ponds, although on occasions some production runs were made specifically with the objective of stocking a pond. The numbers of larvae stocked by pond type is shown in Table 2.5. Some of the pond types are pictured in Figure 2.8.

Figure 2.8: Some of the pond types used for jungle perch larval rearing. From left to right, covered 225 m^2 pond, uncovered 225 m^2 pond and uncovered 1600 m^2 pond.

Table 2.5: Number of jungle perch larvae stocked by pond type

Pond surface area m^2	Covered	Number of larvae stocked
225	yes	1 000 000
225	yes	19 100
225	no	202 000
225	no	726 000
225	yes	169 000
225	yes	128 825
225	yes	136 480
225	yes	1 700 000
225	no	164 800
225	no	177 800
1600	no	98 000
1600	no	511 000
1600	no	189 300
1600	no	241 000
1600	no	146 140
1600	no	259 800
1600	no	229 950
1600	no	436 284
5000	no	229 000

Pond management

All ponds were filled with raw seawater. A 300 µm sock was placed on the inlet filter to prevent fish larvae and fish eggs from entering the ponds, while allowing various zooplankton and phytoplankton to enter the ponds. A mixture of organic and inorganic fertilisers was added to the ponds to promote phytoplankton and zooplankton blooms. Prior to filling, pond beds were scattered with lime or dolomite. Fertilisers were then applied on the dry pond bed, or alternatively as the pond began to fill. Aerators or paddlewheels were used to mix fertilisers through the ponds before any larvae were introduced. Initial liming and fertiliser application rates are shown in Table 2.6. After filling, follow up fertiliser was added to the ponds twice per week at a lesser rate to maintain plankton blooms (Table 2.6). It was normally between three and four weeks after filling that jungle perch larvae were introduced to ponds. This generally provided enough time for a zooplankton bloom to develop.

Zooplankton blooms were monitored by taking 2 L water samples once or twice per week (and more frequently during early larval development) from the ponds. Water was collected in a 2 L Nalgene bottle and was usually collected near an aeration point from approximately 50 cm below the surface. Samples were concentrated to 200 mL by filtering the sample through a 20 µm screen.

Table 2.6: Pond fertiliser application rates

	Pond 15 m x 15 m (225 m^2) 400 000 L		Pond 40 m x 40 m (1600 m^2) 3.2 million L		½ ha pond 50 m x 100 m 10 million L	
Fertilizer	**Initial**	**Follow up twice weekly**	**Initial**	**Follow up twice weekly**	**Initial**	**Follow up twice weekly**
Monoammonium phosphate (kg)	0.143	0.024	1.14	0.19	3.563	0.594
Urea (kg)	0.712	0.119	5.7	0.95	17.813	2.968
Potassium nitrate (kg)	0.645	0.107	5.26	0.876	16.438	2.688
Pollard or bran (kg)	1.25	0.25	10	2	31.25	6.25
Lucerne chaff (kg)	1.25	0.25	10	2	31.25	6.25
Lime or dolomite (kg)	5	-	40	-	120	-

A pipette was used to take six 1 mL subsamples from the concentrated 200 mL sample. Prior to taking each sample the concentrated sample was mixed by blowing gently down the pipette. The 1 mL subsamples were placed in wells for viewing under a dissecting microscope. The different families (and where known, genera and species) of zooplankton were counted in each subsample. Copepods were also counted by stage (nauplius, copepodite and adult copepod). A mean count per 1 mL of concentrated sample was calculated. When multiplied by 100 this gave an estimate of the numbers of different zooplankters per L in the pond. If zooplankton densities were low, six 5 mL subsamples were viewed. The mean count for different zooplankton groups obtained from 5 mL subsamples when multiplied by 20 also gave an estimate of number of zooplankters per L.

Pond water quality parameters, including pH, salinity, temperature, oxygen concentration and Secchi depth were monitored daily. Ponds were aerated by a cluster of air stones set on one side of each pond. Additional aeration and pond circulation were provided by airlifts. Airlifts were made of 100 mm PVC pipe approximately 1.8 m in length. An elbow was attached to the top of the pipe. An air-stone set at the base of the pipe lifted a flow of aerated water up the pipe and it was directed out by the elbow. The air lifts were set in the four corners of the ponds and were all set to direct water in a clockwise direction around the pond. Air-lifts were not normally activated at early larval stages (unless O_2 levels were low) and generally were activated after larvae were more than seven days post hatch. At this stage the larvae were considered robust enough to handle the currents generated. Paddlewheels were not used once larvae were introduced to a pond. This was to avoid damage to buoyant yolk sac larvae and larvae in general. Observations of tank held larvae suggested they gathered near the surface to feed. It was thought that paddlewheels could easily kill larvae that clustered near the surface, and it was also believed that the very small jungle perch larvae may struggle to feed in the vigorous currents generated by paddlewheels. Initial aeration in ponds was provided by the cluster of air stones set on one side of the pond. This ensured adequate oxygenation, but also left some current-free zones in the ponds for early-feeding larvae.

In the first two seasons pond plankton blooms were natural, however, as more was learnt about jungle perch larval requirements, some ponds were seeded with tank-cultured *P. crassirostris* and other copepod species captured by plankton-net from prawn ponds on site. This was to try to increase the copepod nauplii densities at the time jungle perch larvae were released into some of the ponds. Other ponds were left with natural blooms, some with high numbers of rotifers and low numbers of copepods and others with reasonable blooms of copepods. One nursery pond (225 m^2) had purchased sea salt added to it to bring the salinity at time of stocking up to at least 32 ppt. This followed dilution by heavy rainfall. In years one and two of the project none of the pond salinities were manipulated.

Ponds were topped up from time to time with raw seawater to counter evaporation and seepage. Rainfall generally prevented salinities getting too high. The small ponds had water levels set by screened stand pipes. Large ponds had water levels set by a drop board system set behind a 2 mm mesh screen.

Addition of Alpine Blue dye (described earlier) could not prevent filamentous algae from establishing on the walls of the pond within 50 cm of the surface. These algae were mechanically removed as required by winding the strands of algae around wooden stakes. Sea lettuce or *Ulva* also established on the top layer of the pond. This was removed by hand or scooped out by dip net.

Supplementary feeding was introduced to all ponds if larvae were still present 20 days post hatch. Supplementary feeding consisted of larval weaning diets (Ridleys barramundi dust and the O. Range and NRD smallest sized larval weaning diets). In season three, live *Artemia* were also stocked for one week in a 225 m^2 pond at a rate of 53 million nauplii per day for one week. At the same time larvae were weaned onto commercial diet preparations. Artemia were added because fish were required for re-stocking experiments and numbers of adult copepods were declining in the pond during the weaning period. One 425 g container of *Artemia* cysts produces approximately 250 000 *Artemia* nauplii per gram.

Opportunistic samples of larvae were taken from the ponds from time to time for measurement and examination of development with the aid of an inverted light microscope (Nikon Eclipse Ti) at either 20 x or 40 x magnification. Images were captured with a microscope mounted Nikon DS-Fi2 camera and saved using NIS Elements 4.0 software.

Harvesting of fingerlings

When fingerlings had reached 25-30 mm fork length (FL), they were drain harvested into a trap. In large ponds, draining was completed by gradually removing monk boards to reduce the water level in the pond. When the pond dropped to the level of the final monk board, the screen was removed and the final monk board was removed. Water flowed via a 500 mm pipe into a pit in which a trap was set. The entrance of the trap was attached via a shade cloth funnel to the pipe. The funnel led to a box shaped trap mounted on a galvanised iron frame. The outer shell of the trap was constructed of rigid oyster mesh. The inner layer of the trap was constructed of shade cloth to reduce damage to fingerlings. The outlet drains of the pit were dammed with cement besser blocks to raise the water level in the trap to 30-40 cm depth.

Harvest from small ponds followed a similar procedure: water was drained by replacing the usual 150 mm diameter PVC standpipe with a standpipe containing screened drainage holes. Screening was provided by 4 mm oyster mesh or 30% shade cloth. When pond water level had dropped to approximately 25 cm depth near the base of the stand pipe, the standpipe was removed and pond water drained via a 150 mm outlet to a pit where the same trap system as describe above was used (Figure 2.9).

Fingerlings were dip-netted from the trap with soft mesh aquarium nets (Figure 2.9) and transferred to 20 L buckets approximately 30% filled with seawater. At regular intervals these were hauled up from the pit on ropes (bucket lid in place) and transferred to an 800 L fish transporter adjacent to the pit

(Figure 2.10). The fish transporter was filled with UV treated 1 µm filtered seawater. Oxygen was gently diffused into the transporter tank.

After all fingerlings were captured from a pond, they were taken to the hatchery for transfer to tanks. Fingerlings were dip-netted from the fish carrier and transferred into buckets of seawater. Total numbers captured were estimated by weighing several subsamples of individually counted fingerlings added to tared buckets of seawater. Total numbers were then estimated based on the total weight of fingerlings added to buckets of seawater prior to transfer to rearing tanks. Tank based rearing of fingerlings is covered in Chapter 3.

Figure 2.9: Drain harvesting jungle perch fingerlings in a pit trap. Note the raised the water level in the pit achieved by damming outlet drains with besser blocks.

Figure 2.10: Placing drain harvested fingerlings into a fish transporter.

Adaptive management

Pond rearing was essentially run as an adaptive management exercise. Data collected from each season was used to inform how larval rearing may be improved the following season. A key objective was to produce sufficient larvae for reintroduction experiments. As noted above, in the final season some ponds were manipulated to increase densities of copepods and to increase salinities. In some ponds timing of spawning was adjusted according to prevailing pond conditions. For example, a spawn was delayed until a pond had copepod nauplii densities reach a desired threshold density. In other cases larvae were stocked into ponds with poor copepod blooms, or high rotifer blooms. This variation was used to help confirm or reject hypotheses on what factors may be influencing larval survival. The broad range of pond conditions trialled was suitable for multiple regression modelling.

Larval development observations

As outlined above, sub-samples of larvae were taken from tanks during the various larval rearing experiments, and additional specimens were taken opportunistically from ponds. In those tanks where feeding was observed to have taken place, it was considered reliable to use those larvae for analysis of growth and development up until at least seven days post hatch. Development beyond seven days could also be observed in larvae from some of the tanks and in pond reared larvae. Tank rearing temperatures were usually set at 28 ºC. Pond temperatures were more variable, and ranged from 19.2 to 31.4 ºC in the ponds that successfully produced fingerlings. In ponds, variations occurred between day and night and over the rearing period. However, for the majority of the time, temperatures were normally between 26 ºC and 30 ºC. Given fluctuating temperatures, development of larvae from ponds can be expected to be more variable. Sub-sampled larvae were photographed with a Nikon DS-Fi2 camera mounted on a Nikon Eclipse Ti inverted light microscope. Images were saved using NIS Elements 4.0 software. The lengths of larvae were recorded using the NIS Elements 4.0 measurement tool. Key developmental stages were recorded from the images.

Statistical analyses

All statistical analyses were run using the software GenStat™ 16th edition.

The bowl-based experiments examining effects of different variables on larval survival (background colour x lighting x broodstock diet and temperature x larval diet) were analysed by repeated measures ANOVA. The two trial datasets for the background colour x lighting x broodstock diet experiment were combined, with month of the trial treated as a factor in the ANOVA. The variate survival was entered as a percentage into the analyses. The larval diet experiment was analysed by a GLM of binomial proportions followed by a post hoc pairwise comparison.

The preliminary tank-based experiment on salinity and lighting effects on survival used a repeated measures ANOVA as an approximate overall test to analyse the data. In this case lux was entered into the model as a covariate to overcome the unbalanced design. An alternative approach was to group light levels into groups4-21 lux, 90-190 lux and 540-810 lux to create a balanced design for a repeated measures ANOVA and this method of analysis was also used.

The preliminary swim-bladder inflation experiment was run as a generalised linear model (GLM) of binomial proportions, with a logit link function for both larval survival and swim-bladder inflation. A post hoc LSD test on back transformed means was run to examine pairwise differences between days post hatch for larval survival and swim-bladder inflation.

The effect of tank size on larval survival was evaluated using a GLM of binomial proportions with a logit link function followed by a post hoc LSD test on back-transformed means. Interactions between days post hatch and tank size were included in the model. The comparison of larval survival and swim-bladder inflation for larvae reared under upwelling and light aeration conditions was also analysed by a GLM of binomial proportions with logit link function followed by a post hoc LSD test on back-transformed means to detect pairwise differences between treatments and days post hatch. The effect of lighting levels on larval survival and swim bladder inflation was also analysed by a GLM of binomial proportions with a logit link function. Interactions between lighting and days post hatch were considered in the model followed by a post hoc LSD test on back transformed means to examine pairwise differences between treatments across different days. The effect of blue background and granite background on larval survival was also analysed using the same statistical method.

Where appropriate, data from some of the above experiments were displayed as line or bar graphs to indicate some patterns in the data. Given the lack of replication in the opportunistic copepod feeding trials, the data are examined descriptively and plotted as line graphs to indicate trends.

The influence of biological and physicochemical variables on production of fingerlings in ponds was analysed by simple linear regression and stepwise multiple linear regression. The dependent variable was Log_{10} (Fingerlings produced +1). Production numbers were log normalised due to a large number of zero results from ponds, especially in the first year of production. Stepwise multiple linear regression selected from the choice of explanatory variables using an automated procedure. Forward selection starts with no variables in the model and adds a variable that improves the model the most. The stepwise procedure run in GenStat ™ 16th edition involved both forward selection and backward elimination whereby a combination of testing for variables to be added or excluded from the model takes place each step. A variable is always added in the preliminary step. The stepping can continue until no improvement to the model is possible. However, the number of steps used in the model is in part, up to the discretion of the researcher. If adding additional variables to the model explains more of the variance but does not appear to have any biological meaning, the researcher may choose to terminate the model at that point. Explanatory variables run in the simple linear regressions or made available to the stepwise regression model are listed in Table 2.7.

Larval growth was modelled by both simple linear and exponential regression. No statistical analyses were conducted for larval development but each of the key developmental stages and their observed range of timing were described.

Table 2.7: Variables included for use in the stepwise multiple linear regression procedure

Variable	Notes
Number of larvae stocked	
Open pond	Covered =0; Uncovered =1
Pond area m^2	
Salinity at first feed	
pH at first feed	
Oxygen level at first feed	
Temperature at first feed	
Minimum oxygen	
Minimum temperature	
Maximum temperature	
Maximum pH	
Minimum pH	
Minimum salinity	
Maximum salinity	
Copepod density at first feed	
Copepodite density at first feed	
Copepod nauplii density at first feed	
Copepod duration at ≥50 copepods L^{-1}	Number of continuous days where the density exceeded 50 or more per litre post first feed
Copepodite duration at ≥50 copepodites L^{-1}	Number of continuous days where the density exceeded 50 or more per litre post first feed
Copepod nauplii duration at ≥50 nauplii L^{-1}	Number of continuous days where the density exceeded 50 or more per litre post first feed
Rotifer density at first feed	
Mollusc larvae density at first feed	Mostly sea slug larvae but some other gastropod larvae were sometimes present
Ciliate density at first feed	
Tintinnid density at first feed	Tintinnids are a particular group of ciliates
Dinoflagellate density at first feed	
Persistent dinoflagellate bloom	0=no; 1=yes
Secchi depth at first feed	
Maximum Secchi depth	
Minimum Secchi depth	
Hydra infestation	0=no 1=low 2=moderate 3=high

Results

Larval bowl experimental results

Temperature and larval diet

In the larval diet and temperature multifactorial experiment there was no evidence of feeding by larvae on either ss strain rotifers or rock oyster trochophores. No feeding behaviour was observed. There was no significant difference between diet treatments and temperature was not significant as a stand-alone factor. Time was significant, with survival declining with progression of time. There was a significant interaction between temperature and time. Larvae held at lower temperatures had a tendency to survive for an extra day for each 3ºtemperature increment below 31 ºC. The statistical results are summarised in Table 2.8. All larvae held at 31 ºC died by four days post hatch, all larvae held at 28 ºC had perished by five days post hatch and all those held at 25 ºC had died by six days post hatch. Visual observations suggested larvae were more active at 28 ºC and 31 ºC than at 25 ºC on days two and three post hatch.

Table 2.8: Summary of repeated measures ANOVA for the variate per cent survival over time (days 0, 2, 3, 4 and 5) with the factors Temperature and larval diet.

Day 0 is the day of hatch. Day 1 is from 24 hours after hatch, day 2 is from 48 hours after hatch and so on.

Source of variation	Degrees of Freedom	ss	ms	v.r.	F pr.
Subject stratum					
Temperature	2	3 586.3	1 793.1	2.17	0.132
Larval diet	1	497.5	497.5	0.60	0.444
Temperature.Larval diet	2	2 753.5	1 376.7	1.66	0.206
Residual	30	24 824.3	827.5	2.48	
Subject.Time stratum					
d.f. correction factor 0.6402					
Time	4	102 717.1	25 769.3	76.87	<0.001
Time.Temperature	8	17 948.3	2 243.5	6.72	<0.001
Time.Larval diet	4	1 873.3	468.3	1.40	0.251
Time.Temperature.Larval diet	8	3 363.2	420.4	1.26	0.290
Residual	120	40 089.2	334.1		
Total	179	197 652.7			

Background colour, lighting intensity and broodstock diet treatment

In the multifactorial background colour, lighting intensity and broodstock diet experiment, lighting and broodstock diet had significant effects on larval survival. Background colour was not statistically significant at the 5% level. Background colour had no significant interactions with other factors. There was no interaction between month and lighting but there was a significant interaction between month and broodstock and between month and broodstock diet and lighting. Time had a significant effect on survival and time also interacted significantly with all other factors except background colour. A summary of the repeated measures ANOVA is shown in Table 2.9.

After exogenous feeding commenced three days post hatch, survival was higher for larvae in brighter light. Of those larvae in bright light, the tendency was for larvae derived from broodstock on the standard diet to survive better than those derived from broodstock on the terrestrial invertebrate diet (Figure 2.11).

Table 2.9: Summary of repeated measures ANOVA for the variate per cent survival over time (days 0, 1, 2, 3, 4, 5, 6, 7 and 8) with the factors month, broodstock diet, lighting and background colour. Day 0 is the day of hatch. Day 1 is from 24 hours after hatch, day 2 is from 48 hours after hatch and so on.

Source of variation	Degrees of freedom	ss	ms	v.r	F pr.
Subject stratum					
Month	1	98.0	98.0	0.40	0.531
Diet	1	16 282.6	16 282.6	65.78	<0.001
Lighting	1	6 438.4	6 438.4	26.01	<0.001
Background	3	405.9	135.3	0.55	0.652
Month.Diet	1	31 098.7	31 098.7	125.64	<0.001
Month.Lighting	1	589.6	589.6	2.38	0.126
Diet. Lighting	1	3 572.3	3 572.3	14.43	<0.001
Month.Diet.Lighting	1	2 887.8	2 887.8	11.63	<0.001
Residual	85	21 039.5	21 039.5	1.62	
Subject.Time stratum **d.f. correction factor 0.4868**					
Time	8	595 105.6	74 388.2	487.82	<0.001
Time.Month	8	38 555.1	4 819.4	31.60	<0.001
Time.Diet	8	17 392.1	2 174.0	14.26	<0.001
Time.Lighting	8	11 461.9	1 432.7	9.40	<0.001
Time.Background	24	6 117.0	254.9	1.67	0.074
Time.Month.Diet	8	26 163.4	3 270.4	21.45	<0.001
Time.Month.Lighting	8	4 315.4	539.4	3.54	0.008
Time.Diet.Lighting	8	3 238.7	404.8	2.65	0.034
Time. Month.Diet.Lighting	8	2 753.3	344.2	2.26	0.065
Residual	680	103 693.5	152.5		
Total	863	891199.3			

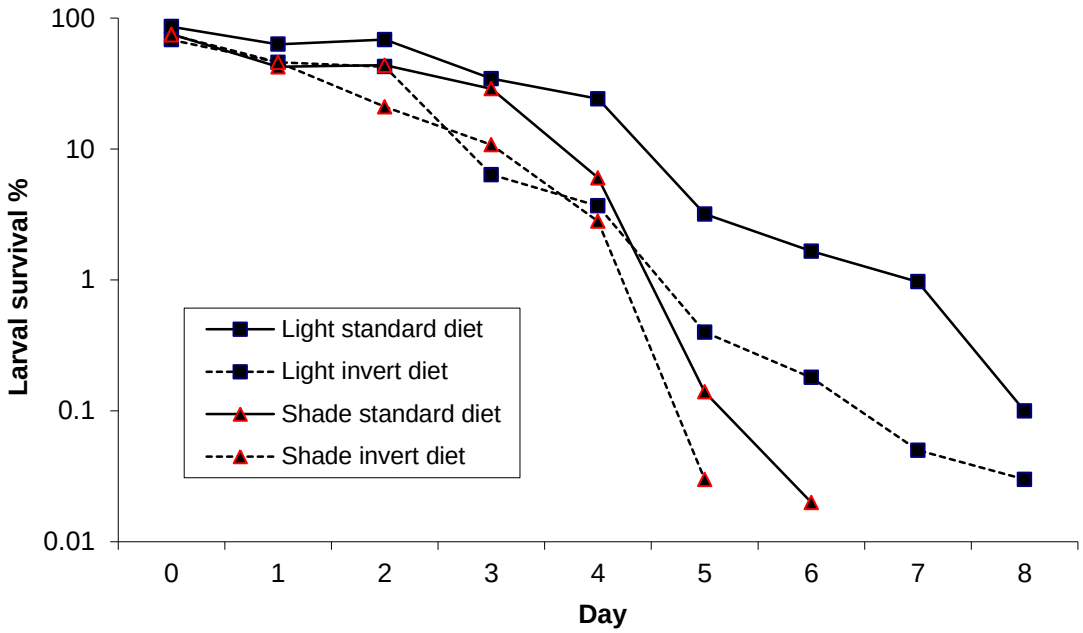

Figure 2.11: Mean larval survival from day 0 (day of hatch) to day 8 post hatch in bowls under light or reduced light under shade cloth. Larvae were sourced from broodstock fed standard diets or invertebrate diets. The Y axis is on a logarithmic scale to help show separation of mean values at survival levels less than 1%. Larvae were maintained on copepod nauplii.

A comparison of three larval diets

Unfortunately this experiment was confounded by the failure of a heater in a trough between three and four days post hatch. This lowered the temperature to between 25 and 26 ºC from the preferred 28 ºC for a subset of some treatments. This may have prolonged survival on endogenous food sources. The analyses of these data must therefore be treated with some caution. This experiment was unable to be repeated due to time constraints. The data are presented here to provide some information on feed preferences of larvae.

Table 2.10 summaries the GLM of binomial proportions. The regression model was run for the period three days post hatch to seven days post hatch, the period when exogenous feeding would be expected to have occurred. The model was significant. The estimates of parameters table for the model is not presented, but days post hatch were significant to the model, with each day post hatch having significantly different survival with reference to day three post hatch ($p<0.05$). Larval feed was not significant, but there was a significant interaction effect between days post hatch and larval diet ($p<0.05$). Post-hoc pairwise comparisons using an LSD procedure are shown in Table 2.11.

It is interesting to note that although there were generally no significant differences between treatments after day five post hatch, it was only in bowls with copepods supplied as a feed where any larvae survived to day seven post hatch (two days beyond other treatments). Some strike postures related to feeding behaviour were observed in some bowls containing copepods. Larvae in copepod bowls appeared to be more active than larvae in other feed treatments from day four post hatch. Numbers of larvae were low in the bowls with surviving larvae on days six and seven post hatch, and some copepod treatment bowls had no surviving larvae. Standard errors were such that despite some evidence that larvae may have been feeding on copepod nauplii and out-surviving larvae in other treatments there was no statistical validation.

Table 2.10: Summary of analysis for the GLM model of binomial proportions for larval diets (copepods, oyster trochophores and ss strain rotifers) over days 3-7 post hatch.

	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx. F pr.
Regression	14	17117	1226.95	28.03	<0.001
Residual	105	4595	43.77		
Total	119	21773	182.96		

Table 2.11: Pairwise tests for mean survival of jungle perch larvae on different days post hatch and fed different larval diets. Analyses were run form days 3 to 7 post hatch, when exogenous feeding would be expected to occur. Means sharing a letter post-script in common are not significantly different to each other at the 0.05 probability level. Note means are as a proportion of 1 (e.g. 0.0150 =1.5% surviving).

Larval diet	Days post hatch	Mean survival
Copepod	3	0.8681a
Copepod	4	0.0150b
Copepod	5	0.0506b
Copepod	6	0.0050b
Copepod	7	0.0025b
ss strain rotifer	3	1.0000c
ss strain rotifer	4	0.4188d
ss strain rotifer	5	0.3112d
ss strain rotifer	6	0.0000b
ss strain rotifer	7	0.0000b
Oyster trochophore	3	0.8800a
Oyster trochophore	4	0.2844d
Oyster trochophore	5	0.0694b
Oyster trochophore	6	0.0000b
Oyster trochophore	7	0.0000b

Results of tank based trials and experiments

Light levels and salinity

Figure 2.12 shows a comparison of survival rates for jungle perch larvae at different salinities between day of hatch (day 0) and five days post hatch (day 5). No feeding behaviour was observed. After day three when exogenous feeding should normally commence (day 3) survival would also be affected by starvation. Survival appears higher at a salinity of 35 ppt, compared to 28 ppt. There were also some differences in survival between lighting groups (Figure 2.13). Very early survival appears better in the low light groups, but beyond day three, survival is poor in all lighting groups.

Light levels and salinity were significant factors ($p<0.05$) in the repeated measures ANOVA (Table 2.12) with light as groups, but there was no interaction between light levels and salinity. Time had a significant effect and there were interactions between time and lighting. The interaction between time and salinity was not significant at the 5% probability level ($p=0.065$). Where light level was used as a covariate in the repeated measures ANOVA (Table 2.13), salinity remained a significant factor and the covariate was also significant ($p<0.05$). Time was significant, representing declining survival with each day post-hatch, but there was no significant interaction between time and salinity ($p=0.113$).

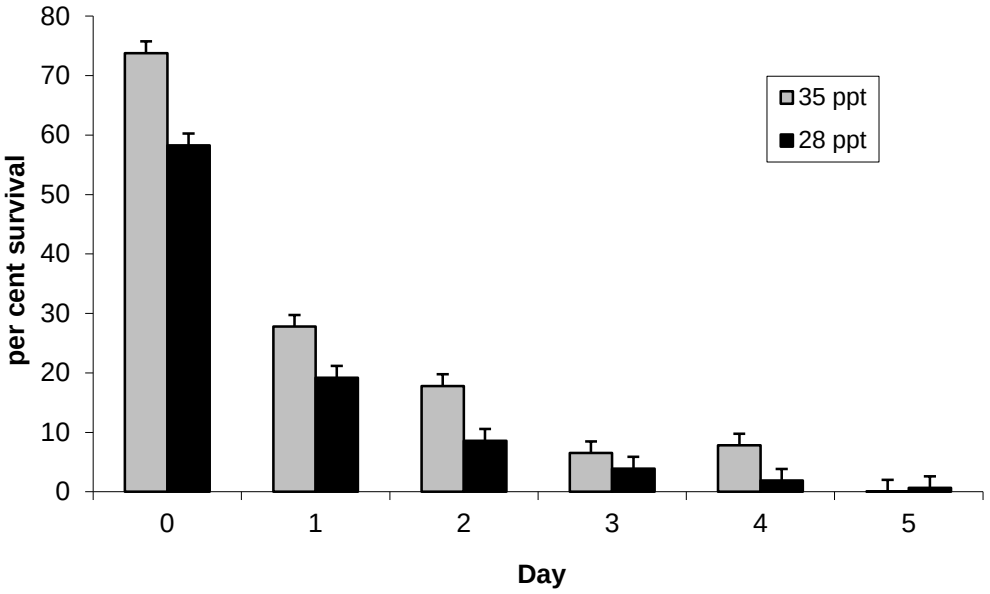

Figure 2.12: Survival of jungle perch larvae at different salinities (28 ppt and 35 ppt) from day of hatch (Day 0) to five day post hatch (Day 5). Note larvae did not feed. Error bars show one standard error of the mean. Note calculations of survival are based on catches of larvae in sub-samples.

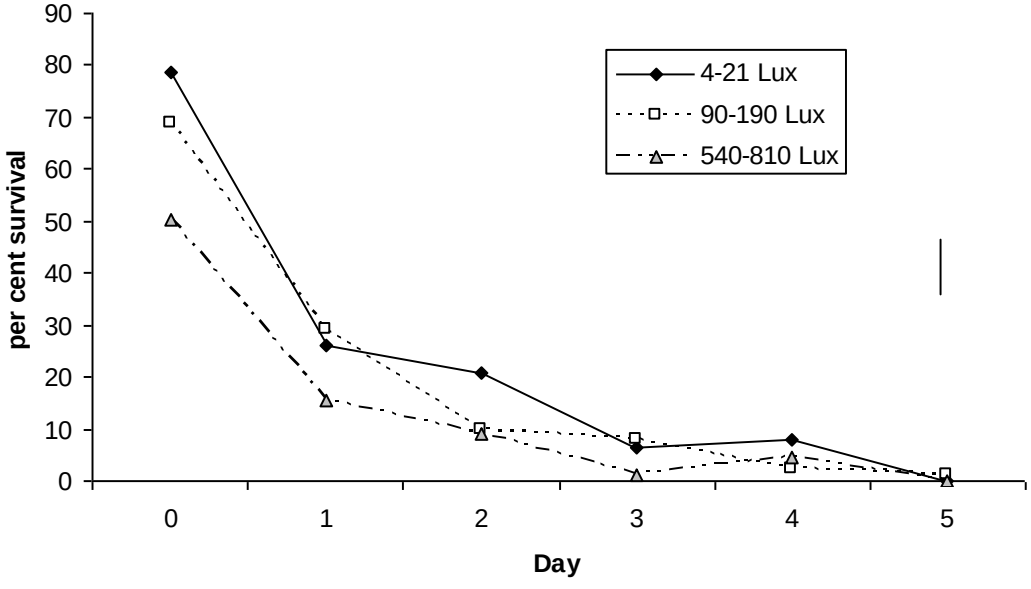

Figure 2.13: Survival of jungle perch larvae at different light levels (4-21 lux, 90-190 lux and 540-810 lux) over time. Note larvae did not feed. Calculations of survival are based on catches of larvae in sub-samples.

Table 2.12: Summary of repeated measures ANOVA for the variate per cent survival of jungle perch larvae over time (days 0, 1, 2, 3, 4 and 5) with the factors salinity and lux group. Day 0 is day of hatch.

Source of variation	Degrees of Freedom	ss	ms	v.r.	F pr.
Subject stratum					
Salinity	1	1 701.42	1 701.42	2.17	0.030
Lux group	2	2 436.07	1 218.04	0.60	0.038
Salinity.Lux group	2	697.02	348.51	1.66	0.324
Residual	12	3 374.67	281.22	2.48	
Subject.Time stratum **d.f. correction factor 0.5851**					
Time	5	72 097.68	1 419.54	201.36	<0.001
Time.Salinity	5	950.82	190.16	2.66	<0.065
Time.Lux group	10	2 745.32	274.53	3.83	0.005
Time. Salinity.Lux group	10	922.83	92.28	1.29	0.288
Residual	60	4 296.65	71.61		
Total	107	67 635.67			

Table 2.13: An approximate overall test for the effect of salinity and lighting on larval survival, using a repeated measures ANOVA, with lux as a covariate to overcome an unbalanced design. The variate is per cent survival of jungle perch larvae over time (days 0, 1, 2, 3, 4 and 5) with the factor salinity. Day 0 is day of hatch.

Source of variation	Degrees of Freedom	ss	ms	v.r.	cov. ef.	F pr.
Subject stratum						
Salinity	1	1 437.83	1 437.83	5.44	1.00	0.034
Covariate	1	1 804.36	1 804.36	6.83		0.020
Residual	15	3 961.19	264.08	3.01	1.36	
Subject.Time stratum **d.f. correction factor 0.6266**						
Time	5	52 281.92	10 456.38	119.31	1.00	<0.001
Time.Salinity	5	908.11	181.62	2..07	1.00	0.113
Residual	80	7 011.32	87.64		1.00	
Total	107	67 635.67				

General observations suggested that one day post hatch, jungle perch larvae tended to be photo-negative. Those larvae in brighter light tended to rest on the bottom in the clear water culture tanks. By day two after hatch and on day three after hatch larvae appeared more photopositive.

Opportunistic copepod feeding trials

1000 L tank trial

Many of the larvae in the 1000 L tank trial had perished by four days after hatch (possibly from aeration buffeting in the small volume), but some larvae persisted until seven days after hatch. The temperature in the tank was approximately 27.5 ºC. Therefore by day five without any feeding it

would be expected no larvae would remain. This suggested some feeding had taken place and had extended survival of some larvae.

Large tank trials

In the March large-tank trial, adult copepod numbers declined to 0.2 adults.mL^{-1} by day 2. The highest density copepod nauplii density achieved in this tank was 0.84 nauplli.mL^{-1}, but on most days densities ranged between 0.2-0.5 nauplii.mL^{-1}. Following stocking of ss strain rotifers, densities did not exceed 0.33 rotifers.mL^{-1}.

Jungle perch larvae declined from 31 000 to 2400 by day three post hatch (Figure 2.14). On day three post hatch, larvae appeared to be very active. By day four, only 2000 larvae were remaining. Larvae continued to decline until only a very small number were left by day eight. Numbers were too low for sub-sampling after day four, and were estimated by observation of the whole tank. Some larvae were observed in the classic S strike feeding posture. Normally at 28 ºC no larvae were left alive by day five after hatch; therefore it was likely some larvae had fed. Furthermore, larvae viewed under the microscope were more developed than those seen previously and some had bulging guts.

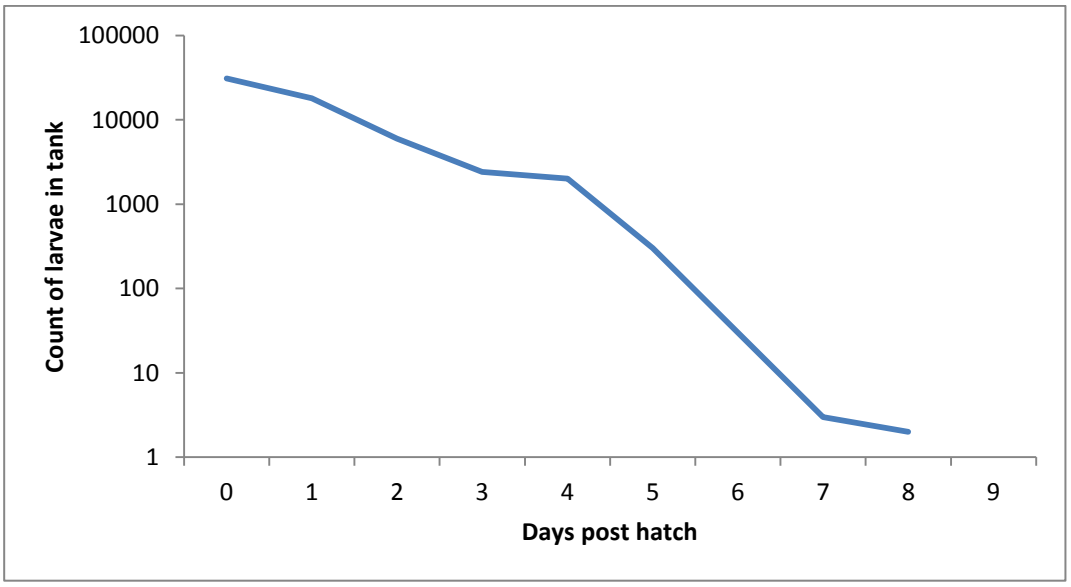

Figure 2.14: Survival of jungle perch larvae in a large tank (filled to 6000 L) stocked with copepods and supplemented with ss strain rotifers stocked at 1 mL^{-1} on day 3 post hatch. Copepod nauplii density ranged between 0.33 and 0.84 mL^{-1}. The y axis is on a logarithmic scale to show low larval numbers more clearly.

In the April trial, tank larvae declined from 21 000 on day of hatch (day 0) to 10 000 larvae by day two post hatch. By day three (day of expected first feed) larval densities remained steady at approximately 10 000. At this stage adult copepods had declined to 0.2 adults.mL^{-1}, but nauplii were present at 2 nauplii.mL^{-1}. Further adult copepods were stocked on day three at 1 adult.mL^{-1}.

By day six an estimated 300 larvae remained. As this was beyond day five it was assumed the remaining larvae must have been successfully feeding and active feeding behaviour with S strike postures was observed. On day six nauplii had declined to 0.67 nauplii.mL^{-1}, but copepodites were present (1 per mL) as were also adult copepods (1.67 per mL). Small-strain rotifers were stocked at a rate of 1 rotifer.mL^{-1}. On day seven approximately 200 larvae remained but nauplii had declined further to 0.2 nauplii.mL^{-1}, and adult copepods had dropped to 0.6 adults.mL^{-1} and copepodites to 0.26 per mL. Further ss rotifers were added at 1 rotifer.mL^{-1} and then again on day eight. By day eight, 200 larvae were still present, but all stages of copepods continued to decline. Approximately 200 larvae were still present and actively swimming on day 10. The decline in numbers of larvae is shown in Figure 2.15. Further adult copepods were added to the tank on day 10 at a rate of 1 adult.mL^{-1}.

On day 13 an estimated 50 to 100 larvae were still present. Adult copepod densities were 0.25 adults.mL^{-1} and nauplii and copepodite densities were both around 0.13 per mL. Small strain rotifers were added at 4 rotifers.mL^{-1}. Some larvae had reached an estimated 4 to 5 mm length by this stage, but the majority were 3.2 to 3.4 mm long. At hatch, larvae in this batch were all less than 2 mm in length.

After overnight flushing between days 13 and 14, ss strain rotifers (6 rotifers.mL^{-1}) and Isochrysis were added early in the morning. There were few if any copepods left in the tank. An estimated 50 larvae survived in the tank. On day 15 only four larvae remained in the tank and all remaining larvae were large (4-5 mm). It appeared most, or possibly all larvae had failed to transition to ss rotifers. No larvae survived beyond day 16.

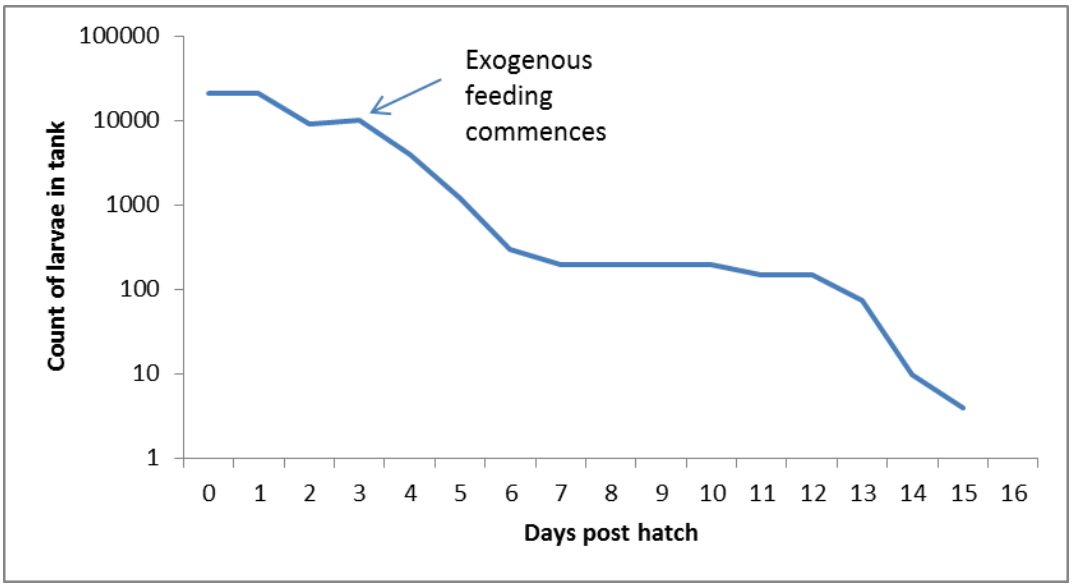

Figure 2.15: Survival of jungle perch larvae in a large tank (filled to 6000 L) stocked with copepods and supplemented with ss strain rotifers stocked at 1 rotifer.mL^{-1} on days 7 and 8 and at 6 rotifers.mL^{-1} on day 14 post hatch. From day 3 to day 13 copepod nauplii densities ranged between 0.13 and 3.3 per mL, before dropping to 0 per mL after day 13. The y axis is on a logarithmic scale is to show low larval numbers more clearly.

Preliminary swim-bladder inflation experiment

In this experiment swim bladder inflation was seen from day five post hatch, reaching 90% of larvae with inflated swim-bladders by day seven post hatch. From day five on there was limited drop off in larval densities, suggesting the increasing proportion of larvae with inflated swim bladders was related to actual increases in inflation rates, rather than to mortality of larvae with un-inflated bladders (Figure 2.16). The peak in larval densities seen on day three post hatch is artificial (Figure 2.16). This was due to aggregating behaviour of larvae near the surface and it was difficult to avoid capture of surface larvae in the beaker samples. During the course of this experiment copepod nauplii densities ranged from 1.33 to 6.67 per mL. At first feed nauplii densities were approximately 5 nauplii.mL^{-1}.

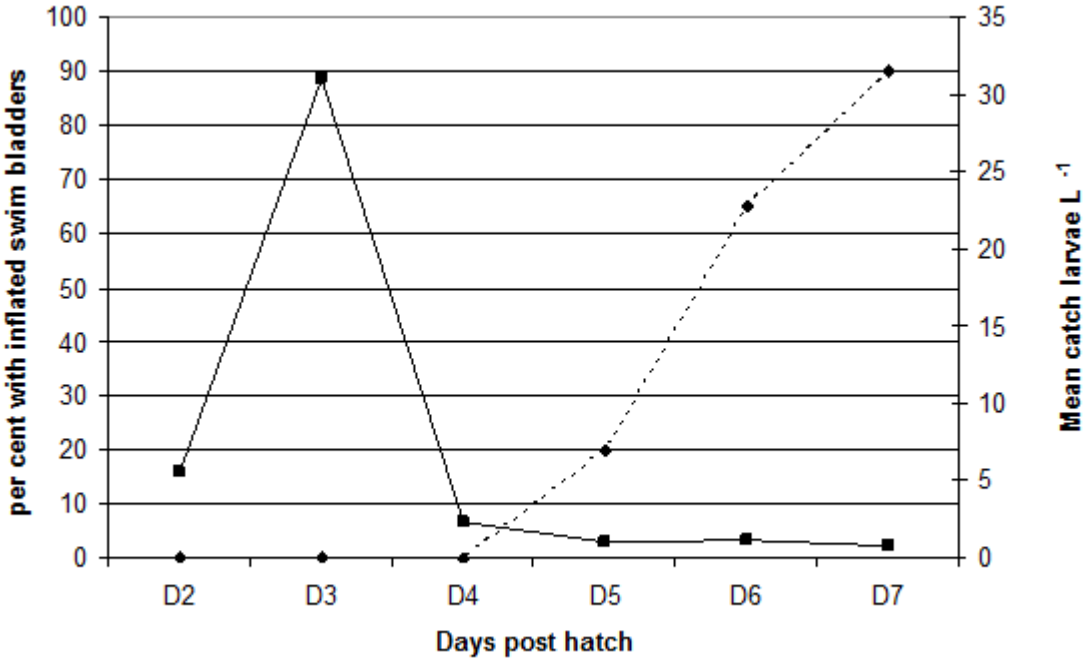

Figure 2.16: Percentage of jungle perch larvae with inflated swim bladders (dashed line) from a lightly aerated tank filled to 3500 L volume. Tank diameter was 3.1 m. Photo-period was 16 h light, 8 h dark. Jungle perch larvae L^{-1} are shown as a solid line. Day 3 density samples are skewed due to aggregating behaviour of larvae near the surface. Day 0 stocking densities were 20 larvae per litre. Larval densities remained fairly stable during the period that increasing percentages of inflation occurred.

Swim-bladder inflation and larval survival in large tanks and small tanks

Swim-bladder inflation was observed as early as four days post hatch in the large tanks, but not until six days post hatch in small tanks (Figure 2.17). Swim-bladder inflation rates were greater in large tanks than in small tanks. The GLM model of binomial proportions and the post hoc pairwise LSD tests showed this difference to be statistically significant (Tables 2.14 and 2.15). However, the swim bladder inflation rates were lower in this experiment than those recorded in the preliminary swim bladder inflation experiment above (Figure 2.16). This may have been related to a different batch of larvae or different copepod densities. Survival of larvae in the small tanks was possibly slightly higher than survival of larvae in the large tanks (but not at a statistically significant level). There was no statistically significant difference between large tank and small tank densities of larvae on any given day due to large variances (Figure 2.18), but there were some limited between day differences as shown by the GLM of binomial proportions and the post hoc pairwise LSD test (Tables 2.16 and 2.17). From day three larvae tended to cluster or school in patches near the surface. This led to big variations in catch rates between sub-samples. It was too difficult to collect sufficient larvae from the large tanks on day seven after hatch to evaluate swim bladder inflation rates. Copepod nauplii densities remained below 1 nauplius.mL^{-1} across all tanks for the duration of this experiment.

Table 2.14: Summary of regression analysis of binomial proportions with logit link function for swim-bladder inflation rates in different tank sizes over seven days.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx. F pr.
Regression	9	34.885	3.8728	22.4	<.001
Residual	9	1.556	0.1729		
Total	18	36.412	2.0229		

Table 2.15: Post hoc LSD test for swim-bladder inflation rates (as a decimal fraction) in different tank sizes over days 2 to 6 post hatch. Means with the same subscript are not significantly different at the P=0.050 level

Day	Tank size	Inflation rate
2	Large	0.00a
2	Small	0.00a
3	Large	0.00a
3	Small	0.00a
4	Large	0.10b
4	Small	0.00a
5	Large	0.10b
5	Small	0.00a
6	Large	0.40c
6	Small	0.15b

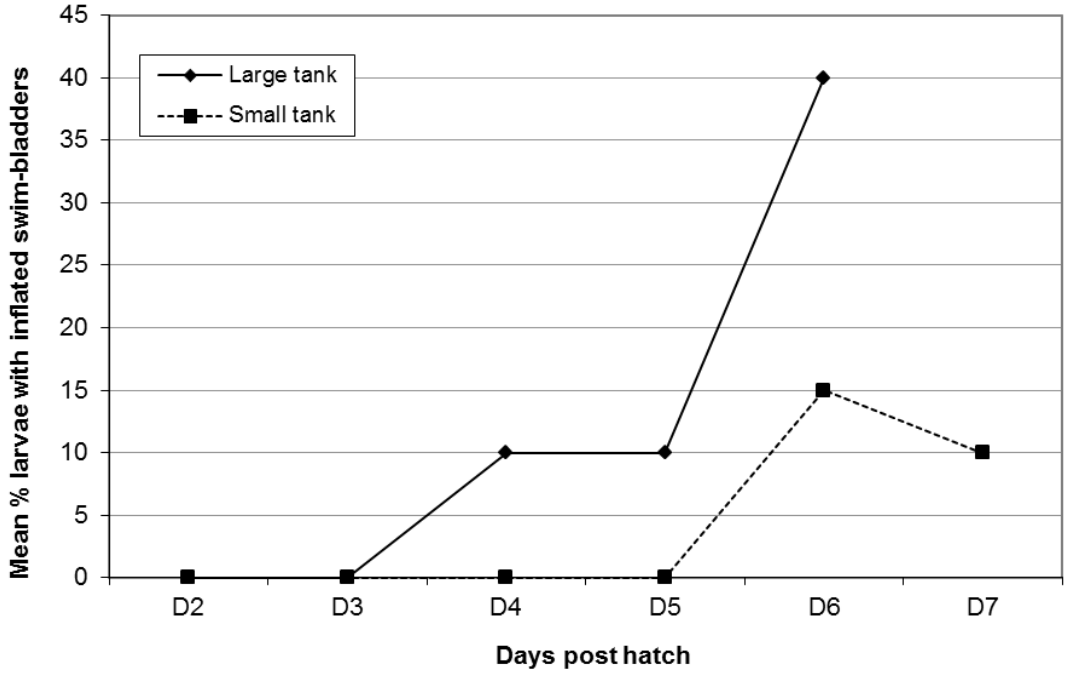

Figure 2.17: Mean percentage of larvae with inflated swim bladders from lightly aerated large and small tanks Copepod nauplii densities were <1 mL^{-1}. Larvae from large tanks are indicated by a solid line, larvae from small tanks by a dashed line.

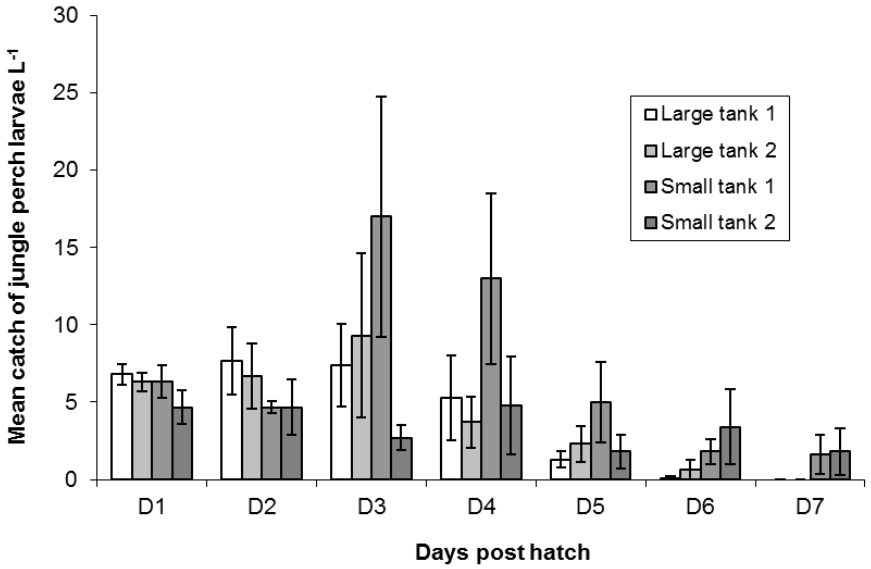

Figure 2.18: Mean catch rates of jungle perch larvae within lightly aerated large and small tanks between 1 and 7 days post hatch. Day 3 and 4 results are biased by aggregating behaviour of larvae near the surface. Error bars show ± one standard error of the mean. Day zero stocking densities were 22.5 larvae per litre. Some larvae remained in the large tanks on day 7 but were not collected in random samples.

Table 2.16: Summary of analysis for a GLM of binomial proportions with logit link function for larval survival rates in different tank sizes over seven days.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx. F pr.
Regression	13	83.36	6.412	2.91	0.029
Residual	14	30.86	2.204		
Total	27	114.22	4.230		

Table 2.17: Post hoc LSD test for larval survival (as a decimal fraction) in different tank sizes across 7 days post hatch. Means with the same subscript are not significantly different at the P = 0.050 level. Note survival estimates are based on catch rates of larvae in 2 L subsamples. These may have been biased by clustering of larvae.

Day	Tank size	Larval survival rates
1	Large	0.2911ab
1	Small	0.2444ab
2	Large	0.3196ab
2	Small	0.2076ab
3	Large	0.3711ab
3	Small	0.4378a
4	Large	0.2000ab
4	Small	0.3956ab
5	Large	0.0800ab
5	Small	0.1511ab
6	Large	0.0156ab
6	Small	0.1156ab
7	Large	0.0000b
7	Small	0.0756ab

Lighting levels

There was a significant effect of lighting on survival. In this experiment the GLM of binomial proportions was significant (Table 2.18). There were significant interactions between lighting and days post hatch. There were also significant differences in densities of larvae between day one and day eight in the lit tanks and significant differences in larval densities between day one and days five, six, seven and eight post hatch in the ambient lighting tanks (Table 2.19) as shown by the post hoc pairwise testing. Note that differences took longer to emerge in the lit tanks. There were also significant differences in densities of larvae between lit and unlit (ambient) treatments from day six onwards. Illuminated tanks (2500 lux) had higher survival than tanks under ambient hatchery lighting (150 lux). By day six there was total mortality in the ambient lighting tanks, whereas numerous larvae were remaining in illuminated tanks (see Table 2.19). There was no swim bladder inflation recorded from the ambient lighting tanks (Figure 2.19), but 100% of examined larvae in illuminated tanks had inflated swim bladders on day eight. It is probable that there was mortality of the majority of larvae without inflated swim-bladders by day eight. Inflation began to be observed from day five after hatch in illuminated tanks.

Table 2.18: Summary of analysis for GLM of binomial proportions with logit link function for survival of jungle perch larvae. Fitted terms constant, days post hatch, light and days post hatch.light. Note "days post hatch.light" is an interaction term.

	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx. F pr.
Regression	15	148.05	9.8699	13.67	<0.001
Residual	16	11.56	0.7222		
Total	31	159.60	5.1485		

Table 2.19: Comparison of larval survival in lit (2500 lux) and ambient (150 lux) tanks between 1 and 8 days after hatch.

Lighting	Days post hatch	Mean proportion of larvae recorded relative to stocking rate	Significance (means with the same subscript are not significantly different at the 5% level)
2500 Lux	1	0.3225	ab
2500 Lux	2	0.2000	abcd
2500 Lux	3	0.1500	abcd
2500 Lux	4	0.2812	abc
2500 Lux	5	0.2425	abcd
2500 Lux	6	0.3550	a
2500 Lux	7	0.1250	bcd
2500 Lux	8	0.1050	cde
Ambient	1	0.3100	abc
Ambient	2	0.1875	abcd
Ambient	3	0.1650	abcd
Ambient	4	0.1550	abcd
Ambient	5	0.0600	de
Ambient	6	0.0000	e
Ambient	7	0.0000	e
Ambient	8	0.0000	e

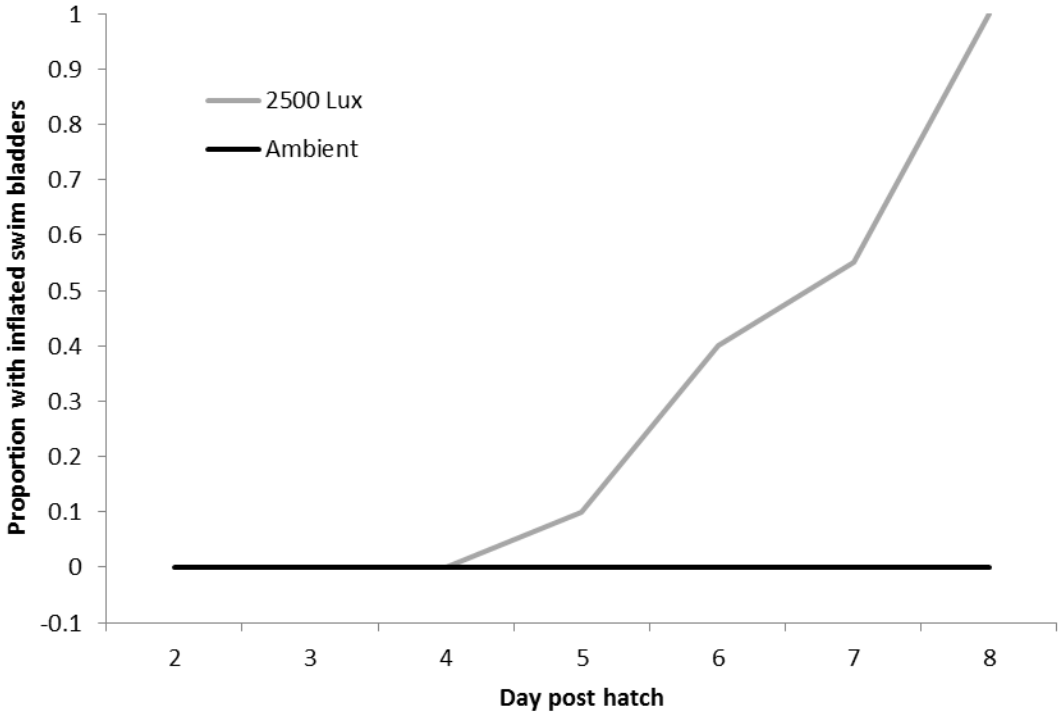

Figure 2.19: Mean swim-bladder inflation rates between 2 and 8 days after hatch in lit tanks (2500 lux) and tanks with ambient lighting (150 lux). Note from day 6 there was no survival of larvae in the tanks with ambient lighting. Larvae in tanks with ambient lighting did not inflate their swim-bladders.

Gentle aeration and upwelling systems

Larvae were observed to behave very differently in upwelling systems and lightly aerated systems. In the upwelling system larvae congregated closely against the sides of the tank. In the aeration system larvae were more evenly spread through the tank, but favoured the brighter side. By day four no larvae were still alive in the upwelling tanks, none were captured in sub-samples and none could be observed in the tanks. Larvae persisted and were actively feeding in the light aeration systems. Analysis of survival rates using a GLM of binomial proportions showed there were significant effects (Table 2.20) and there were statistically significant differences in survival rates between upwelling and light aeration systems by three days post hatch. See the post hoc test of pairwise differences in Table 2.21. As noted above, no survivors were detected in upwelling systems from day four onwards (Figure 2.20, Table 2.21,). Despite the upwelling tanks having no larvae on days six and seven, the pairwise test showed no significant difference between survival in lightly aerated and upwelling tanks. This was due to high levels of variance of larval samples from the lightly aerated tanks resulting from clustering of larvae. Clearly survival was better in the lightly aerated tanks, with an estimated 7.56% of larvae remaining seven days after hatch in lightly aerated tanks.

Typically at 28 ºC, if no feeding has occurred, then all jungle perch larvae perish 4 days post hatch. Copepod nauplii densities remained similar in all tanks from both treatments, at just under 1 nauplius.mL^{-1}.

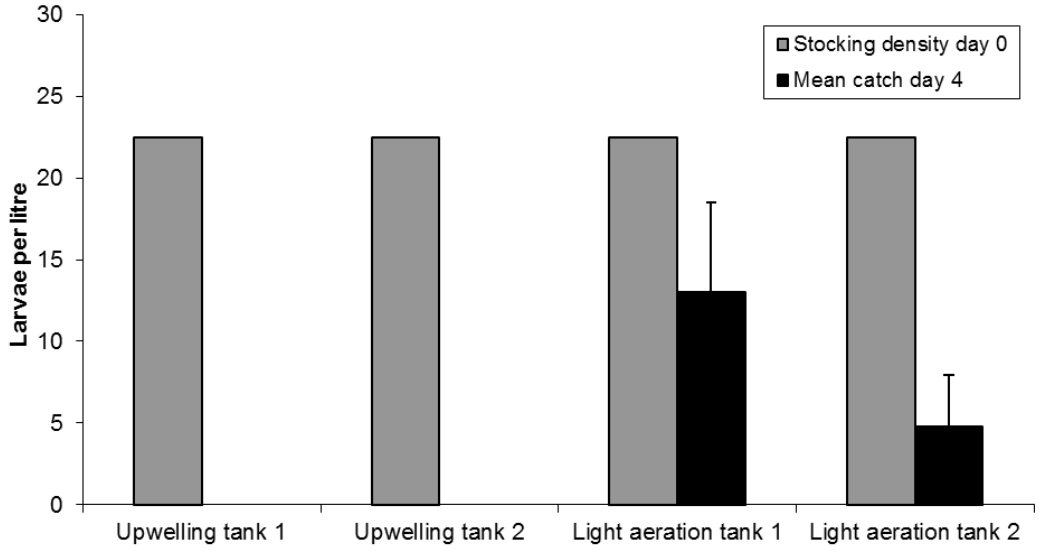

Figure 2.20: Survival of larvae at 4 days post hatch in upwelling tanks and tanks with light aeration compared to the original stocking density. Error bars show one SEM (n=5). Temperature was set at 28 ºC.

Table 2.20: Summary of regression analysis of binomial proportions with logit link function for larval survival rates in different tank with upwelling and light aeration systems over seven days.

Source	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx. F pr.
Regression	13	110.97	8.536	3.72	0.010
Residual	14	32.09	2.292		
Total	27	143.06	5.299		

Table 2.21: Post hoc LSD test for larval survival in upwelling and light aeration systems across 7 days post hatch. Means with the same subscript are not significantly different at the P = 0.050 level

Day	Tank size	Survival rate
1	Light	0.2444abc
1	Upwelling	0.2667abc
2	Light	0.2076abc
2	Upwelling	0.1258acd
3	Light	0.4378be
3	Upwelling	0.0518ad
4	Light	0.3956ce
4	Upwelling	0.0000d
5	Light	0.1511abcde
5	Upwelling	0.0000d
6	Light	0.1156acd
6	Upwelling	0.0000d
7	Light	0.0756ad
7	Upwelling	0.0000d

Blue and granite backgrounds

As shown in Table 2.22, the overall regression model for effect of background colour and days post hatch on larval survival was significant ($p < 0.001$). However, background colour itself was not significant, but days post hatch and the interaction term days post hatch.background colour were significant compared to the reference background colour blue and day one ($p < 0.05$).

Mean copepod nauplii densities recorded in the blue tanks and granite tanks on day three post hatch (first day of feeding) were 3.1 and 4.6 nauplii.mL^{-1} respectively, which is adequate for feeding.

As can be seen in Figure 2.21, up until day four post-hatch larval densities in the blue background and granite background tanks were not significantly different (although the mean values tended to be higher in the granite background tanks) and followed similar patterns, but from day five larvae in the granite background tanks declined in density much more rapidly than larvae in blue background tanks. By day five post hatch larval densities in granite background tanks were significantly lower than those on day one (see Table 4). In blue background tanks, larval densities were not significantly different to those on day one, until day seven.

Despite the apparent large difference in densities of larvae between granite background and blue background tanks on days five to seven, there was no statistically significant difference between the granite and blue background tanks on those days. Larvae were clearly more abundant in all the blue background tanks compared to all the granite tanks on those days (i.e. the lowest value sub-sample collected in the blue tanks was higher than the highest value subsample collected in the granite tanks), but the variance was high in the blue background tanks due to the clustering behaviour of the larvae. Therefore the post hoc LSD model did not detect a significant difference (Table 2.23). This was a consequence of low levels of replication, which was dictated by our capacity to produce sufficient copepods to sustain the experiments.

Visual observations did suggest fewer jungle perch larvae were aggregating near the edges of the tank in the granite lined tank, but this did not confer a survival advantage. Observations of jungle perch larvae in blue tanks suggested some larvae were consuming copepods or copepod nauplii trapped in the meniscus around the edges of blue tanks.

Table 2.22: Summary of analysis for count of jungle perch larvae per 2 L. Fitted terms constant, days post hatch, background colour and days post hatch.background colour. Note "days post hatch.background colour" is an interaction term.

	Degrees of freedom	Deviance	Mean deviance	Deviance ratio	Approx. F pr.
Regression	13	113.20	8.708	6.05	<0.001
Residual	14	20.14	1.439		
Total	27	133.35	4.939		

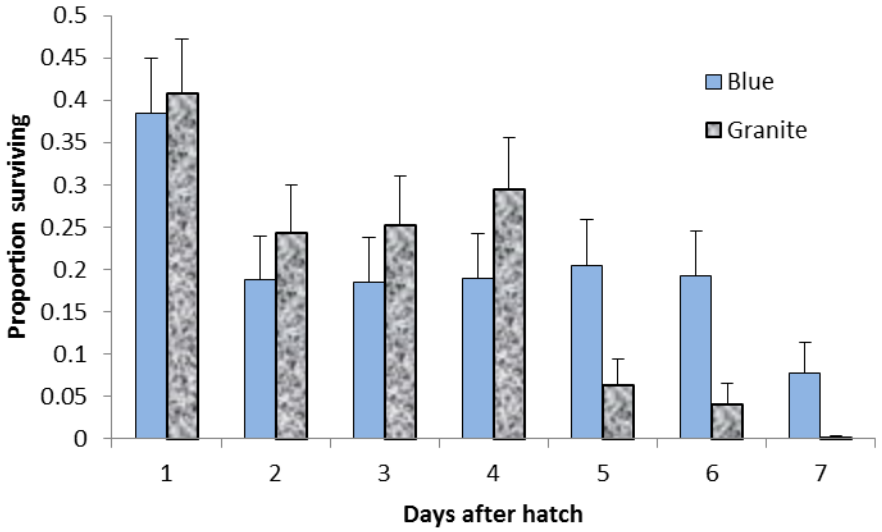

Figure 2.21: Proportion of larvae surviving (based on counts per 2 L sample) compared to initial stocking density in tanks with light blue and granite background colours. Error bars show one standard error of the mean.

Table 2.23: Comparison of larval survival in blue background and granite background coloured tanks between 1 and 7 days after hatch.

Lighting	Days post hatch	Mean proportion of larvae recorded relative to stocking rate	Significance (means with the same subscript are not significantly different at the 5% level)
blue	1	0.3850	a
blue	2	0.1875	abc
blue	3	0.1850	abc
blue	4	0.1900	abc
blue	5	0.2050	abc
blue	6	0.1925	abc
blue	7	0.0775	bc
granite	1	0.4075	a
granite	2	0.2425	ab
granite	3	0.2525	ab
granite	4	0.2950	ab
granite	5	0.0625	bc
granite	6	0.0400	bc
granite	7	0.0003	c

Results of pond trials

In the analyses of pond data by step-wise multiple regression, the first variable selected by stepwise linear regression was "copepod nauplii duration at ≥50 nauplii.L^{-1}". This accounted for 53.6% of the variance and was positively correlated with jungle perch fingerling production. The next variable selected by the model was pH at first feed. This was negatively associated with jungle perch production, suggesting higher pH readings had poorer survival of fingerlings. It should be noted that pH levels in the ponds were not very extreme. Together the variables "copepod nauplii duration at ≥50 nauplii.L^{-1}" and "pH at first feed" accounted for 65.7% of the variance. The final variable added to the model was "minimum pH". This variable also had a negative relationship with jungle perch fingerling

production. This suggests that if the minimum pH level was relatively high, then fingerling output could be expected to be lower. As noted above pH levels recorded in the ponds were not extreme.

The variables "copepod nauplii duration at ≥50 nauplii.L^{-1}", "pH at first feed" and "minimum pH" accounted for 70.1% of the variance. See the summary of analysis table (Table 2.24) and estimates of parameters tables (Table 2.25) below.

Table 2.24: Summary of analysis for the response variate Log_{10}(N)+1 Pond fingerling production. Fitted terms: Constant, copepod nauplii duration at ≥50 nauplii.L^{-1}, pH at first feed and minimum pH.

	Degrees of freedom	s.s	m.s	v.r.	F pr.
Regression	3	19.970	6.6566	15.06.	<0.001
Residual	15	6.615	0.4410		
Total	18	26.585	1.4769		

Table 2.25: Estimate of parameters for the stepwise linear regression. Fitted terms: Constant, copepod nauplii duration at ≥50L^{-1}, pH at first feed and minimum pH. The response variate is Log_{10}(N)+1 Pond fingerling production

Fitted terms	Estimate	s.e.	T (15)	t pr.
Constant	16.87	5.07	3.33	0.005
Copepod nauplii duration at ≥50 nauplii.L^{-1}	0.0843	0.0130	6.50	0.001
pH at first feed	-1.122	0.528	-2.13	0.050
Minimum pH	-0.905	0.493	-1.83	0.087

The number of copepod nauplii at time of first feed was not selected by the stepwise regression process as a significant variable explaining survival of larvae. However, it should be noted that in no cases where numbers of copepod nauplii were <130 nauplii.L^{-1} at time of first feed, did any larvae survive to fingerling stage. However, there were occasions where copepod nauplii numbers were high at time of first feed, but no larvae survived. These were either short-lived copepod blooms, or in ponds that were shortly afterwards affected by heavy rainfall and sudden drops in salinity or crashes in oxygen levels.

The variable "copepod nauplii duration at ≥50 nauplii.L^{-1}" was the only variable significant in simple linear regression. "pH at first feed" and "minimum pH" were only significant in multiple linear models.

Larvae first become readily visible in ponds at seven days post hatch when yellow pigment around the abdominal region becomes prominent. Larvae tended to school near the surface. In mornings larvae were more aggregated on eastern and northern sides of the pond and in the afternoons on western and northern sides of the pond. They appeared to follow the sun. After metamorphosis fry tended to sit deeper than larvae and often aggregated adjacent to aerators or auto-feeders.

Larval growth and development

Growth

Larval growth is best explained by exponential regression (R^2 = 0.902, y = $2.1483e^{0.066x}$). Simple linear regression did not fit the data as well and had a lower R^2 value (R^2 = 0.835 y = 0.3597x + 1.3969). The exponential curve is shown in Figure 2.22.

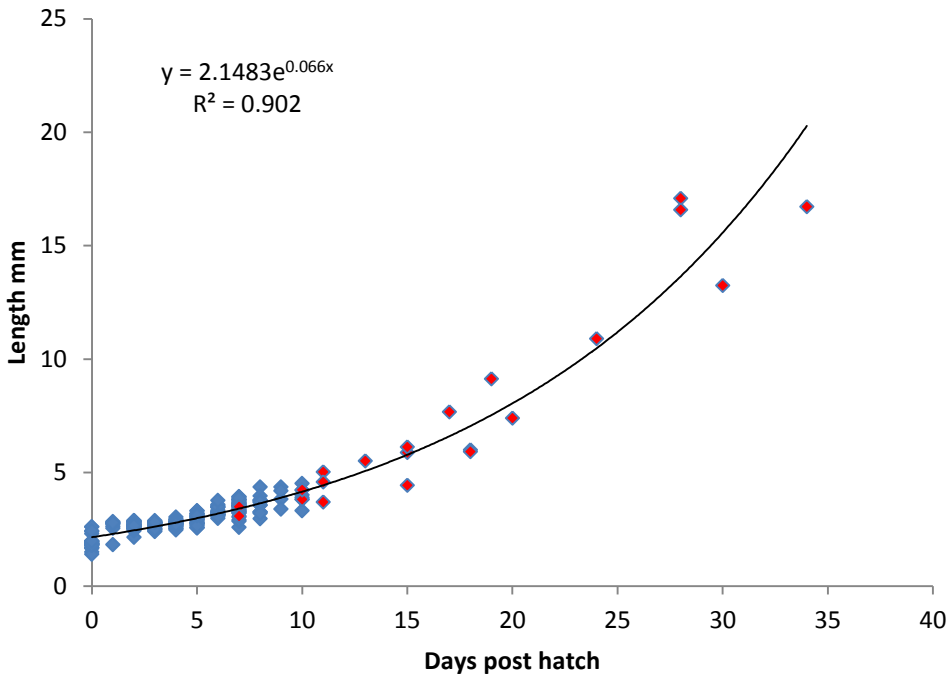

Figure 2.22: Exponential growth curve for jungle perch larvae. Blue points represent tank-derived data and red points represent pond-derived data (n = 229).

Development

The development of jungle perch larvae from newly hatched yolk sac larvae through metamorphosis to fingerlings is shown in Figures 2.23, 2.24 and 2.25. A summary of the key developmental stages is provided in Table 2.26. There was some variation in the timing of the different stages, but this appears to be related to growth rates of individual larvae. This is reflected in Figures 2.23, 2.24 and 2.25. For larvae reared at 28 ºC, feeding commenced at three days post hatch. Swim bladder inflation occurred between four and seven days post hatch. Flexion occurred between 11 and 15 days post hatch. Post flexion commenced from between 16 and 19 days post hatch and squamation (formation of scales) or completion of metamorphosis occurred between 28 and 34 days post hatch at 16-17 mm total length (TL). Fins of fry were fully pigmented when fry were between 25 and 30 mm TL.

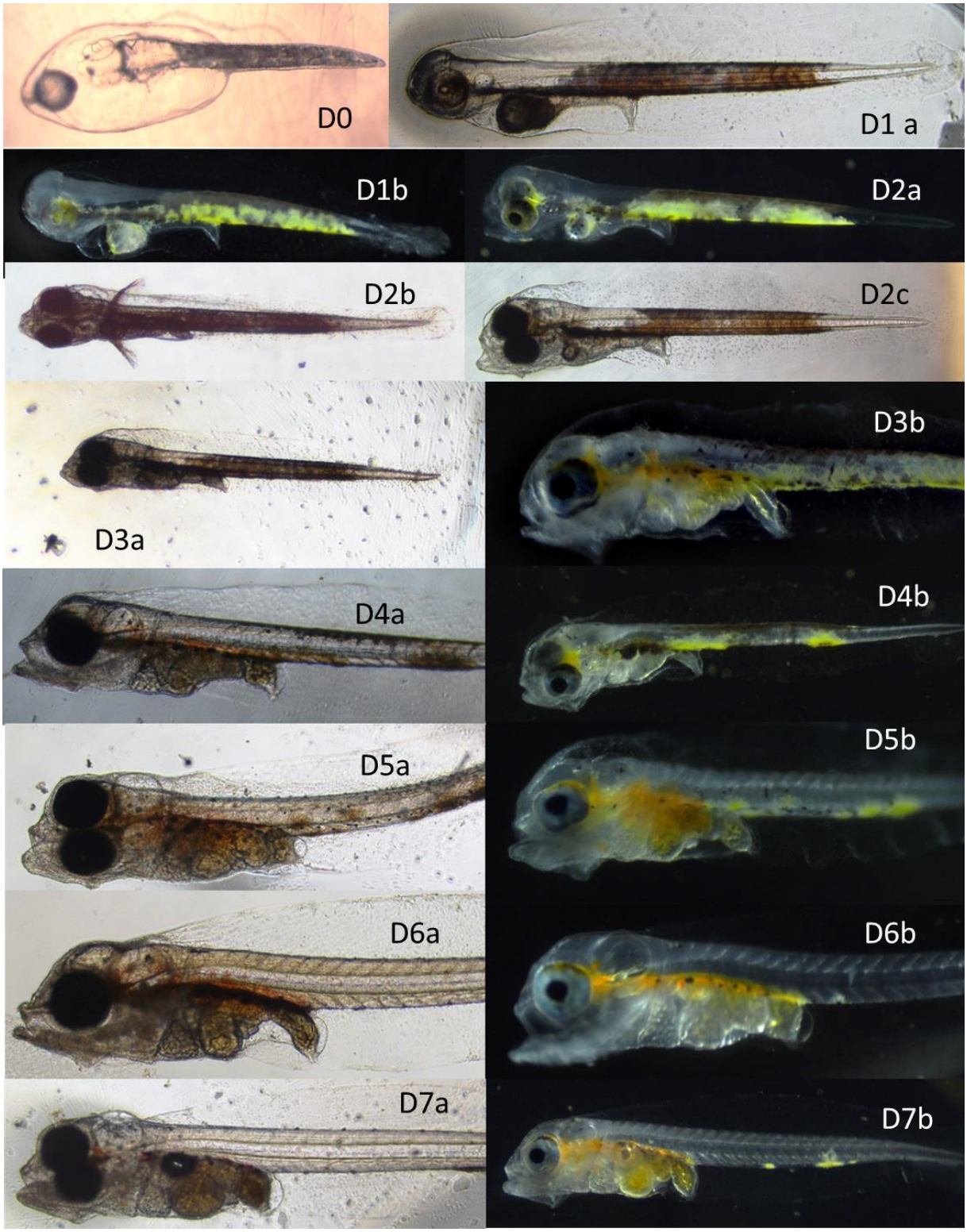

Figure 2.23: Larval development from day of hatch (D0) to 7 days post hatch (D7) at 28 ºC. Each stage includes bright-light high-contrast images to show some internal structures and low-light images to show some external and internal pigmentation. All images show a lateral view of the larvae except for D2b, which shows a ventral view of a larva two days post hatch to display the pectoral fins. Image D3a shows a bright-light high-contrast image of a larva three days post hatch with a copepod nauplii. Swim bladders can be seen in the images of D6 and D7 larvae.

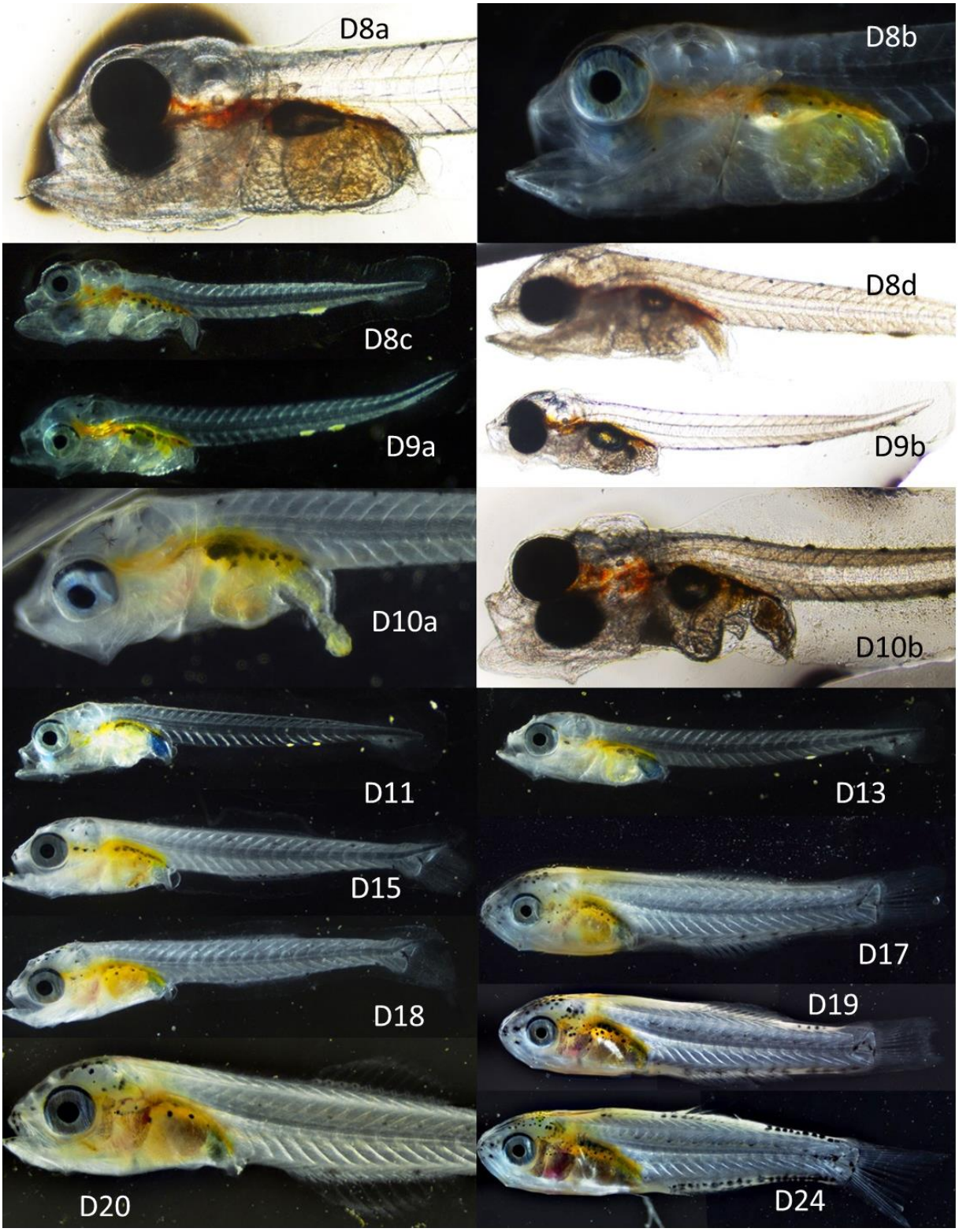

Figure 2.24: Larval development from 8 days post hatch (D8a, D8b, D8c and D8d) to 24 days post hatch (D24). Development to 10 days post hatch is at 28 ºC. Older fish were generally sampled from ponds, generally in the temperature range 26-30 ºC. Until 10 days post hatch, both-bright light high-contrast images to show some internal structures and low-light images to show some external and internal pigmentation are included. From 11 days post hatch (D11) to 24 days post hatch (D24) low light images only are shown. All images show a lateral view of the larvae. D8a and D8b show close up images of the head and gut region of a larva 8 days post hatch with an inflated swim bladder prominent.

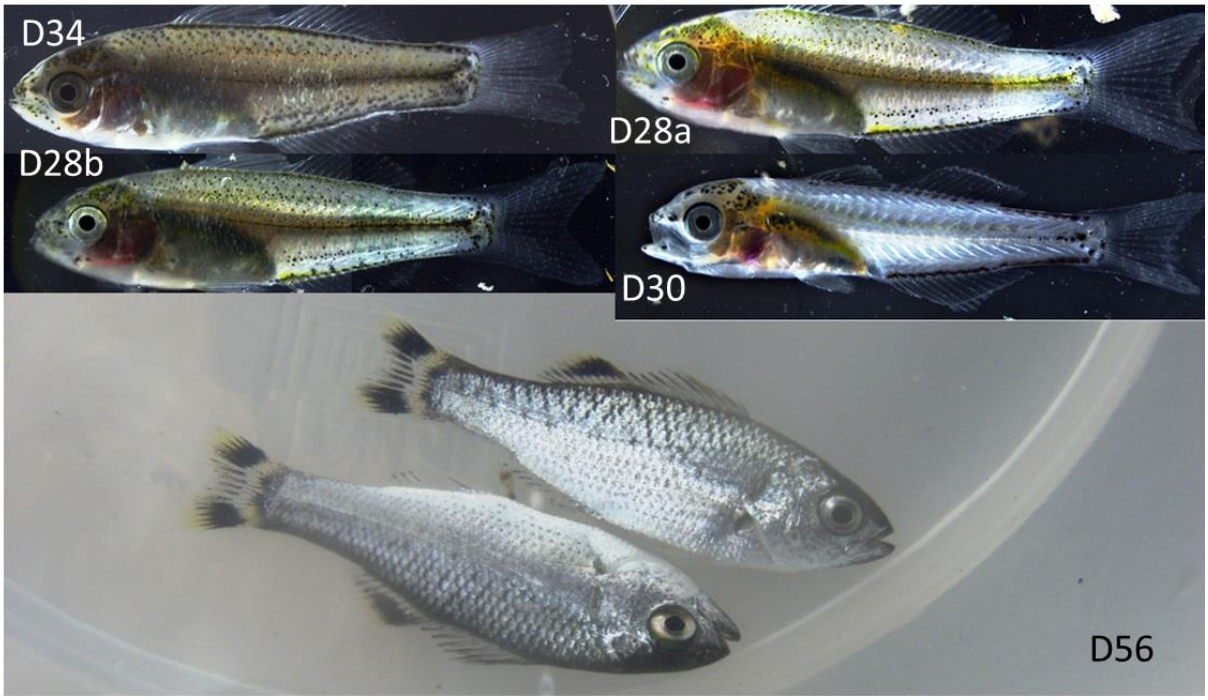

Figure 2.25: Late-stage larvae and metamorphosed fry and fingerlings collected from ponds between day 28 and day 56 after hatch. D34 features a just-metamorphosed fish (squamation has just been completed) 34 days post hatch. D28a and D28b are two individuals at the identical developmental stage to D34, but at 28 days post hatch. All three fish were between 16.5 mm and 17 mm TL. D30 is a late stage post-flexion larva 30 days post hatch, but only 14 mm TL. The D56 fingerlings are 56 days post hatch and 30 mm TL. They have developed the characteristic flag-tailed pigmentation which develops from around 25 mm TL.

Table 2.26: Summary of development of jungle perch larvae and fry. From yolk-sac larvae to 10 days post hatch, observations are based on tank-held larvae reared at 28 ºC. Days 11 to 15 are based on both tank-reared and pond-reared larvae and days 16 onwards are based on pond-reared larvae only.

Developmental stage	Description
Yolk sac larvae day of hatch	This stage occurs on day of hatch (D0). The yolk sac and oil globule are prominent. Length 1.4-2.6 mm. Lens buds present.
1 day post hatch (endogenous feeding)	Most of the yolk has been used. Oil globule still prominent. Anus and simple linear alimentary canal beginning to form. Auditory vesicle present. Eyes forming but not fully functional. Finfold surrounds notochord. Length 1.8-2.8 mm
2 days post hatch (endogenous feeding)	Eyes functional. Pectoral fins developed. Mouth formed. Otoliths visible in auditory vesicle. Alimentary canal continuing to develop. Length 2.2-2.9 mm. Oil globule still present.
3 days post hatch (exogenous feeding commences, pre-flexion)	Eyes prominent. Mouth well formed. Oil globule mostly used up. Exogenous feeding commences. Folds forming in alimentary tract. Stomach present. Liver forming. Length 2.4-2.9 mm.
4 to 7 days post hatch (pre-flexion)	Swim bladder inflation occurs between days 4 and 7 post hatch. Most usually on days 6 or 7 post hatch. Timing may in part be related to rate of growth and generally occurs in larvae >3mm TL. The size range of larvae D4-D7 is from 2.6 to 3.9 mm. The digestive tract continues to develop. Increasing numbers of folds develop in the gut. Liver and spleen become more prominent. Myomeres prominent from 5 days post hatch. Yellow pigment begins to form over the abdominal region, becoming prominent by 7 days post hatch. Operculum beginning to form from day 6 or 7 post hatch. Fin fold undifferentiated
Days 8-10 post hatch (pre-flexion)	Continuing somatic growth. Swim bladders prominent. Operculum developing. Increasing yellow pigmentation over gut region. Melanophores developing over the swim bladder. Notch beginning to form at caudal end of finfold. Size range 3.25-4.5 mm TL
Days 11-15 post hatch (flexion)	Flexion may occur as early as day 11 or as late as day 15 post hatch depending on growth rates. Caudal fin rays begin to develop. Early development of dorsal and anal fin rays commences. Pigmentation over the abdominal region obscures the swim bladder. Size range 3.7-6.1 mm TL
Days 16-19 (post-flexion)	Post flexion may develop as early as 16 days post hatch or as late as 19 days post hatch. Occurs at a size of at least 7.5 mm TL. During this period development and differentiation of pelvic, dorsal and anal fin rays occurs. The caudal fin continues to develop. Melanophores develop over the head and abdomen. Size range 6.2- 9.1 mm TL
Days 20-24 (pre-metamorphosis)	Jaw becomes more underslung. Increasing pigmentation (development of melanophores) over the dorsal and ventral surfaces. Increasing fin differentiation. Operculum well developed. Size range D20-24 = 7.5-10.9 mm TL
Days 25-34 post hatch (metamorphosis and squamation)	Fin and fin ray development continues. Caudal fin becomes more forked. Larvae become increasingly pigmented. Squamation (scale formation) may occur as early as 28 days post hatch or as late as 34 days post hatch. Occurs when larvae reach 16-17 mm TL. Size range of larvae D24-D34 = 11.0-17.1 mm TL.
Days 34-56 post hatch (growth and development of fry)	Post squamation melanophore development continues. Black markings in caudal and dorsal fins develop between 25 mm and 30 mm TL.

Discussion

Larval feeds

Larval bowls were probably sub-optimal for rearing jungle perch larvae, and survival rates were probably poorer in larval bowls than they may have been in tanks, but the bowl based experiments did permit increased replication and did provide some initial insights into factors that may affect jungle perch larval rearing success.

The bowl based trials provided evidence that ss strain rotifers and rock oyster trochophores are not suitable first feeds for jungle perch larvae. The lack of feeding made it difficult to properly evaluate the effect of temperature, but in the early stages of development larvae held at 28 ºC and 31 ºC appeared in better condition and more active than the fish held at 25 ºC. However they used up their yolk-sacs and oil droplets more rapidly and as a consequence died sooner. The activity levels of larvae at 28 C suggested that if offered the appropriate feed they may be more successful at capturing prey than larvae reared at lower temperatures.

The bowl based comparison of three larval diets provided the first suggestion that the copepod (*P. crassirostris*) could be a suitable first feed for jungle perch larvae. Although not statistically significant, it was only in larval bowls where copepod nauplii were offered as a feed that any larvae survived to seven days post hatch. At the rearing temperature of 28 ºC all larvae not offered copepods were dead by six days post hatch.

In the bowl based tank background colour experiment, it was copepod nauplii that were provided as a feed and jungle perch larvae were able to persist in some bowls until eight days post hatch. Further confirmation that copepod nauplii were a suitable first feed and that copepod nauplii and later stages may be able to sustain jungle perch larvae came from opportunistic copepod feeding trials in large tanks, where larvae were sustained for up to 15 days. Further confirmation of the importance of copepod nauplii came from pond rearing trials, where the duration of copepod nauplii blooms at densities above 50 nauplii.L^{-1} was a significant factor in explaining pond fingerling production.

The number of copepod nauplii at time of first feed was not selected by the stepwise regression process as a significant variable for explaining survival of larvae. However, it should be noted that in no cases where numbers of copepod nauplii were less than 130 per L at time of first feed did any larvae survive to fingerling stage. However, there were occasions where copepod nauplii numbers were high at time of first feed, but no larvae survived. These were either short-lived copepod blooms, or blooms in ponds that were affected by heavy rainfall and sudden drops in salinity or crashes in oxygen levels. Most of these were one-off events, so are not readily picked up as significant by statistical models.

We were unable to transition jungle perch larvae from copepods to ss strain rotifer or rotifers. This is one aspect of tank rearing that perhaps should be investigated further. At BIRC we had limited capacity to produce copepods in sufficient numbers for a sustained period of tank rearing, and it is likely many other hatcheries may experience similar difficulties. If jungle perch could be successfully transitioned from copepod nauplii to enriched rotifers in the first week of tank rearing, then tank rearing would become a more attractive prospect for many hatcheries.

Observations of pond conditions suggest that jungle perch larvae will not transition well to rotifers. Ponds with low densities of copepods and high densities of rotifers did not result in fingerling production. The low densities of copepod nauplii in some ponds should have been sufficient to stimulate some feeding behaviour, and if this resulted in a transfer to feeding on rotifers, then at least some fingerlings could have been expected to be produced from such ponds.

If transitioning to rotifers is not an option, then for many hatcheries there would be benefits in focusing efforts on pond rearing. Embryos could be stocked directly into ponds, or larvae could be transferred to ponds after five to seven days rearing in tanks on cultured copepod nauplii. To improve production results, timing of spawning induction should be dictated by the state of the pond plankton

bloom. Copepod nauplii densities of at least 130 nauplii.L^{-1} should be the minimum level at which stocking of larvae should be attempted. At BIRC we have achieved nauplii densities in excess of 900 per L. Chances of successful pond copepod blooms can be increased by seeding the pond at an early stage with *P. crassirostris*.

Although jungle perch larvae were unable to be transitioned to rotifers, they were able to be successfully fed *Artemia* from 20 days post hatch and could begin to be weaned onto artificial diets at this stage. It is likely that jungle perch larvae are stimulated to feed by the jerky swimming motion of copepod nauplii and copepods, as has been noted for other species (Young 1994a,b; Moe 1997; Buskey 2005; Marcus 2005). Based on the evidence from this project, it would appear that jungle perch larvae have larval diet requirements similar to the flame angelfish *C. loricula*, which appears to need copepods for the entire larval life cycle (Schipp 2006). *Kuhlia mugil*, a marine relative of jungle perch *K. rupestris*, readily accepted nauplii of the copepod *Parvocalanus crassirostris* as a first feed and were fed a diet of copepods (*Parvocalanus*, *Acartia* and *Pseudodiaptomus*) and rotifers throughout the larval period, before weaning onto Cyclop-eeze and *Artemia* (Gardner 2013). It is not clear from Gardner's work if jungle perch larvae actually fed on rotifers or not. It appears rotifers were provided together with various copepod species, therefore *K. mugil* larvae may not necessarily have consumed the rotifers. There was no weaning onto rotifers described, only weaning onto *Artemia* and Cyclop-eeze (a freeze dried micro-crustacean). This is very similar to our rearing of *K. rupestris* larvae, where larvae were weaned from copepods to *Artemia* and powdered diets in ponds.

Broodstock diets

The multifactorial larval bowl experiment for background colour, lighting and broodstock diet, suggested broodstock diet had a significant effect on larval survival, and there were significant interactions between lighting and broodstock diet and time, and broodstock diet on the survival of jungle perch larvae. The general trend was for larvae derived from broodstock on the standard diet (comprising mainly marine derived components) to do better than larvae from broodstock on a diet high in terrestrial invertebrate components. In the previous chapter it was noted that the mean fertilisation rate was significantly higher for eggs from the standard diet treatment than from the invertebrate diet treatment. This was linked to elevated dietary levels of HUFA found in the standard diet compared with the invertebrate diet. It is probable that better nutrient provisioning in the yolk and oil globules of larvae from the standard diet treatment contributed to improved survival. During the endogenous feeding phase, larvae from the standard diet treatment may have had a developmental advantage over larvae from the terrestrial invertebrate diet group that assisted them when exogenous feeding commenced.

Background colour and lighting

In the larval bowl multifactorial experiment on background colour, lighting and broodstock diet, background colour was not found to have a statistically significant effect on survival. This is in contrast to results found for some other species, where background colour did have a significant effect on survival (Tamazouzt *et al.* 2000; McLean *et al.* 2008; Opiyo 2010; el-Sayed & El-Ghobashy 2011). Due to the static nature and small volume of the bowls, water quality may have become an issue towards the end of this experiment that could have reduced larval survival and possibly affected the overall result. Testing multiple colour backgrounds in tanks rather than bowls was constrained by the production of sufficient numbers of copepods and copepod nauplii.

Generally jungle perch larvae were able to be reared successfully in sky blue background coloured tanks until copepodite or copepod nauplii densities dropped to low levels. A significant difference in survival was found between tanks with blue coloured backgrounds and granite (speckled) coloured backgrounds. This result was the opposite to what we expected and opposite to the results obtained for striped trumpeter and yellowtail kingfish *(*Cobroft & Battaglene 2009; Yeoman 2014). It is probable that the granite background may have reduced walling behaviour in jungle perch larvae, but this usually beneficial effect may have been negated by reduced feeding efficiency. Perhaps copepod nauplii and copepodites are more difficult to visualise against a granite background and this may have

accounted for the sudden drop in larval density on day five in the granite background tanks. We have observed that copepods tend to aggregate around the surface margins of blue background illuminated tanks (see larval rearing video with the production manual). This may make it easier for jungle perch larvae to target copepod prey. It cannot be discounted that low levels of toxins may have leached from the vinyl granite coloured coating. It is possible jungle perch larvae may be more sensitive to chemicals in vinyl than striped trumpeter and yellowtail kingfish. The only way to resolve this would be to conduct future experiments with both the blue and granite backgrounds being vinyl or to trial tanks painted with a granite pattern.

Lighting had a significant effect on larval survival in all of the experiments where light was a variable. However, in one of the trials involving lighting (the salinity and lighting tank based trial), larvae did not feed because the feed offered was oyster trochophores. In that experiment dull light seemed to confer a survival advantage to larvae early on. However, in the lighting and background colour trials in bowls where larvae were fed copepods, shading or dull light seemed to confer a survival disadvantage.

The 1000 L tanks in the above trial were clear water cultures. Larvae tended to rest on the bottom in the bright tanks on day one, whereas in the dull tanks they were distributed throughout the water column. Settling on the tank bottom may have made larvae more susceptible to bacteria. By day two or three larvae tend to be more photopositive. In the larval bowls the curved bottom of the bowls were less conducive to larvae settling on the bottom, so larvae may have been less susceptible to bacterial infection early on. From day three onwards light may have assisted feeding of larvae in the bowls. Bowl larvae were offered copepods, whereas tank larvae had trochophores. Copepods seemed to be recognised as food, whereas trochophores were not.

There is the possibility that the significance of factors tested as determinants of survival may change if the larvae were grown under conditions where they actually survived. Some factors that appear significant when survival is poor may not be significant under improved rearing conditions. This could well be the case for lighting, where if the right type of feed is offered bright light seems to confer an advantage.

Other tank based trials involving comparisons of larval survival in bright light and ambient light confirmed the trend seen in the larval bowl trials, where larvae exposed to bright light had better survival. In these tanks copepods were offered as a feed and the alga T-Iso was also added to the tanks. T-Iso provided some shading effect, which may have been beneficial to larvae early on. Larvae in unlit tanks generally starved to death, whereas larvae in lit tanks could be seen actively feeding. Larvae tended to congregate on the brighter side of the tank. It is possible that lighting may help aggregate copepods, copepodites and copepod nauplii near the surface or on the brighter side of the tanks. This may confer a feeding advantage to jungle perch larvae in lit tanks over those in unlit tanks. Swim-bladder inflation was also better in lit tanks than in unlit tanks. We suggest this may have also been related to feeding. Efficient feeding by jungle perch larvae in lit tanks probably enabled them to reach a critical size, at which they could break through the surface tension to take a breath and inflate their swim bladders. In tanks where lighting was inadequate and feeding less effective, larvae probably failed to reach the critical size to enable them to successfully inflate their swim-bladders. This is most likely why swim bladder inflation was not seen in tanks with ambient lighting. Failure to inflate a swim-bladder would place further energetic strains on fish larvae, which would further limit their chances of survival.

The link between bright light and feeding behaviour of jungle perch is further supported by observations of feeding larvae aggregating near the surface in pond rearing experiments. Larvae also seemed to follow the daily movements of the sun across the pond. Bright light has been shown to be beneficial to the rearing of other species, including cod *G. morhua* (Monk *et al.* 2006) and yellowtail kingfish (Woolley *et al.* 2012). For successful rearing of jungle perch larvae in tanks, it appears essential to provide bright light and copepods in a green-water rearing system.

Tank size

There was some suggestion from the large and small-tank experiment that large tanks improve swim-bladder inflation rates. However this may only be the case when copepod nauplii densities are low. High levels of swim bladder inflation were observed in small tanks when copepod nauplii densities were high. There appeared to be no survival advantage for larvae in large (3.1 m diameter) tanks over larvae in small (1.1 m diameter) tanks.

Salinity, pH and other physicochemical variables

Salinities close to full seawater appear best for hatching and survival of larvae. (See also the results of salinity experiments in Chapter 1.) There was no interaction between salinity and time. The increased mortality appeared to be fixed at an early stage by lower levels of salinity. It seems likely that lack of buoyancy of yolk-sac larvae at 28 ppt may have contributed to reduced survival by exposing larvae to greater risk of bacterial exposure, by concentrating larvae near to the bottom of the tank. In contrast in full seawater yolk-sac larvae were able to raft near the surface of the tanks.

It is worth noting that although salinity was not selected by the stepwise linear regression model as a significant factor in explaining fingerling production in ponds, none of the ponds with a salinity level of less than 30 ppt at the time of stocking had any jungle perch fingerlings produced. Many of the ponds produced no fingerlings. Low salinity and low oxygen levels may have resulted in non-production of fingerlings in some of the ponds. For example, in one pond oxygen dropped to 1.1 $mg.L^{-1}$ despite aeration. In this pond numbers of jungle perch larvae and available feed had been quite high until the oxygen crash. In two ponds salinity dropped below 26 ppt after heavy rain. Other ponds remained above the critical salinity of 32 ppt and oxygen levels tended to remain above 4 $mg.L^{-1}$. The low oxygen and low salinity events were only one-off and two-off events. Minimum salinity or salinity at time of stocking was not shown by the statistical analyses as being significant. This is probably because other variables may have been more important in the other zero or low output results in other ponds and these may have masked low salinity effects.

Early stage jungle perch larvae appear far less tolerant of reduced salinities than some other diadromous species such as barramundi (Partridge *et al*. 2008), or Australian bass (Fielder & Heasman 2011) or estuarine species like mulloway (Fielder & Heasman 2011). In this regard they are more similar to catadromous eel larvae (Kurokawa *et al*. 2013). However, after metamorphosis jungle perch have a strong preference for freshwater. This suggests that wild jungle perch are much more likely to be oceanic, rather than estuarine spawners. If they do breed in estuaries then it would most likely be in the lower estuary before the full onset of the wet season. Mangrove jack *L. argentimaculatus* larvae were less able to deal with abrupt changes in salinity from an ambient salinity of 32 ppt in the early stages of their development, but with increasing age (i.e. from 0-7 days) they became more tolerant of salinity changes. Tolerances of abrupt salinity changes increased remarkably on day 28 (Estudillo *et al*. 2000). Mangrove jack are offshore spawners, but juveniles and sub-adults of mangrove jack frequently penetrate freshwater streams (Russell *et al*. 2003). It is likely that salinity tolerances of jungle perch larvae may also improve with increasing size and age. Therefore it is possible that salinity levels may only be critical in the first few days after hatch.

pH at first feed was negatively associated with jungle perch production, suggesting higher pH readings led to poorer survival to fingerling stage. It should be noted that pH levels in the ponds were not very extreme. Levels of pH that produced better production of fingerlings are possibly related to conditions that favour production of copepods. “Minimum pH” also had a negative relationship with jungle perch fingerling production. This suggests that if the minimum pH level was relatively high, then fingerling output could be expected to be lower. As noted above, pH levels recorded in the ponds were not particularly extreme, so it is possible that the pH readings may be related to some other aspect of pond productivity that was influencing survival rates of larvae to fingerlings. If pond pH levels started approaching 9.5, we tended to add molasses to the pond to reduce pH levels. However, this can increase oxygen demand, so aeration levels needed to be increased.

Aeration and upwelling

Upwelling resulted in poor survival of jungle perch larvae, which are relatively small around the time of first feed (between 2.4 and 2.9 mm TL). The upwelling seemed to interfere with their ability to feed. In contrast, upwelling has been used successfully with other species including yellowtail kingfish (Fielder & Heasman 2011). Perhaps jungle perch larvae were transported too quickly by the upwelling current to capture prey efficiently. In contrast, the gentle aeration did not seem to impact larvae to the same extent. A single air-stone placed near the central standpipe created water movement, but the standpipe also created a relatively sheltered area in the tank where first feeding larvae could avoid the current. If larvae could feed, then after a few days they would reach a stage where they could cope with currents. Similar observations were made by us of larvae in ponds. Appropriate aeration to prevent excessive turbulence was also found to be important for the growth and survival of *L. lineata* larvae (Shaw *et al*. 2010).

Larval development

The early stages of development are broadly similar to the early development of two other pelagic marine-estuarine spawning diadromous species, barramundi *L. calcarifer* (Shadrin & Pavlov 2015) and sea mullet *M. cephalus* (Abraham *et al.* 1999). Jungle perch larvae rapidly use up endogenous sources of feed compared to many species, therefore exogenous feeding is critical by three days post hatch.

Knowledge of the timing of swim bladder inflation in juvenile jungle perch is important for tank and pond management purposes. Between days four and seven post hatch it is essential that surface skimmers are working efficiently so larvae can push through the water surface to take a breath. In ponds we suggest it is important to refrain from using organic fertilizers such as pollard or lucerne during this period. Lucerne and pollard can float on the surface for some hours, and this can lead to temporary scumming of the surface, which potentially could inhibit swim-bladder inflation. Exposure of the pond surface to breezes helps to keep the surface clear of scum that could inhibit swim-bladder inflation. In ponds covered by greenhouse structures that blocked wind flow, we noticed that pond surfaces readily developed a surface scum, comprising various organics, including microalgae. This may have inhibited swim-bladder inflation and contributed to poor survival. In fact, no larvae developed through to fingerlings in covered ponds. Poor swim bladder inflation may have been one contributing factor.

Adequate feeding also seems to be important for swim bladder inflation. In tanks where copepod nauplii densities were reduced, or where feeding efficiency was reduced, swim bladder inflation rates were poor. Maintaining high densities (>1 nauplius.mL^{-1}) of copepod nauplii seems essential to maximise swim bladder inflation in jungle perch larvae.

Larvae metamorphosed into scaled fry between 28 and 34 days post hatch. Jungle perch fry are known to enter freshwater at 35 to 50 days old at a size of approximately 18 mm TL (Henderson, 2010; Feutry *et al*. 2012; Feutry *et al*. 2013; Hamer *et al*. 2015). Even though jungle perch may be able to tolerate transfer into freshwater at this stage, we recommend delaying harvest until between 50-60 days post hatch, when jungle perch larvae are at least 25 mm TL. Smaller fry have a tendency to faint when handled, but fry above 25 mm TL are very robust to handling, with harvest mortalities less than 1%.

Future research

The current work on jungle perch larval rearing has resolved some of the issues that prevented successful jungle perch larval rearing in the past. Jungle perch fingerlings have been produced for the first time and pond production has been improved to a point where it could be commercially viable. Undoubtedly, further improvements can be made to jungle perch larval rearing. The information provided in this chapter provides a solid foundation for future work. To advance tank production, it would seem that larger scale T-Iso and copepod production is required to enable greater replication of tank based experiments, including further work on tank background colours, and optimal lighting

conditions. Experiments that compare tanks where larvae are attempted to be transitioned to enriched rotifers, with tanks where they are maintained on copepods for more extended periods would also be informative. Investigations into the earliest stage at which jungle perch larvae will wean onto Artemia nauplii may also help progress tank production of larvae.

Other future research options could include investigating optimal densities of copepods and larvae in tanks, such that larvae may more quickly reach a critical size where they can be transitioned to *Artemia* and weaning diets. Further work could also be done to improve the reliability of pond production.

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Chapter 3: Rearing of fingerlings and evaluating stocking success

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Summary

Jungle perch fingerlings were produced in seawater ponds at the BIRC. Fingerlings received pre-release conditioning to live invertebrate feeds and pre-release conditioning to predators. After conditioning, fingerlings were micro-tagged with either visible implant elastomer (VIE) or wire tags. Tagged fingerlings were stocked into Currumbin Creek (900) and the Mooloolah River (864) in south-east Queensland at mean sizes of stock 92.8 mm FL and 51.4 mm FL respectively. Two batches of tagged jungle perch were stocked into St Helens Creek north of Mackay. In November 2014, 42 juvenile fish with a mean FL of 150 mm were released into the creek. In June 2015, 1500 fingerlings with a mean FL of 47.5 mm were released.

These stream systems were selected for reintroduction of jungle perch because they were within the historical range of jungle perch, had adequate (restored) fish passage and significant sections of intact or restored riparian vegetation and substantial areas of what was identified as suitable habitat for jungle perch. Pre-release surveys in all three stream systems detected no jungle perch. However, pre-release surveys did detect adequate numbers of prey fish species and macroinvertebrates to sustain jungle perch fingerlings. All systems had some predatory fish species present and several species of diadromous fish species. Presence of other diadromous fish species suggested that jungle perch should be able to establish self-sustaining populations.

Post-release surveys were conducted in all three systems. Various sampling methods were used, including boat and back pack electrofishing, snorkelling surveys, bankside observations (with the aid of polaroid glasses) and underwater video surveys. Stocked jungle perch were detected in all three systems. Recaptured fish showed evidence of growth. The majority of detections were made upstream of the release sites. Some fingerlings may have moved as much as 10 km upstream. Jungle perch tended to cluster into small patches of habitat, which made detection difficult. Following a major flood in both the Mooloolah River and Currumbin Creek systems in May 2015, no further recaptures or observations of jungle perch were made in these two systems. The flood may have had a detrimental effect on the released fingerlings or dispersed them to areas in the systems that were not sampled.

Ideally, restocking should continue in the three systems over another three to five years, to build up genetic diversity and numbers of jungle perch in the receiving systems. After that period, stocking should cease and monitoring of natural recruitment should commence. It is only through demonstration of natural recruitment that the re-introductions can be deemed successful. If successful, then there may be merit in expanding reintroductions of jungle perch to other restored catchments. If no natural recruitment is detected, then jungle perch need not be reintroduced to other restored river systems.

Introduction

Kuhlia rupestris (jungle perch) is a popular recreational angling target (Allen *et al*.2002; Hutchison *et al.* 2002; Pusey *et al*. 2004). However, jungle perch numbers have declined severely in the southern half of their range in Australia (Hutchison *et al.* 2002). This decline comprises approximately 50% of their Australian range, which would potentially qualify this species as vulnerable if it were to be formerly nominated for EPBC listing. Microsatellite data for northern (Cairns and Daintree region) and southern Queensland (Fraser Island) populations of jungle perch indicated that the two populations are slightly but significantly differentiated (Broderick & Ovenden 2009). Therefore re-establishing jungle perch within their historical range is an important action for conservation of the species. If self-sustaining populations could be re-established, then there is also potential for re-establishment of recreational fisheries for this species within its historical range. Recreational fishing for this species used to exist in catchments such as the Maroochy River and Pioneer River (Hutchison *et al.* 2002), where jungle perch are now very scarce or absent. Much of the angling for this species is catch and release, thus it is unlikely that angling will be detrimental to re-establishment of this species. In fact anglers are the major advocate group for the reintroduction and conservation of this species.

Barriers to fish passage such as weirs and dams are thought to be largely responsible for the decline in jungle perch. Small juveniles would have difficulty negotiating many of the weir structures, even some low weirs (particularly those that do not drown out very frequently) can act as substantial barriers. Jungle perch are capable jumpers, but if there is inadequate depth at the base of a weir, then jumping becomes difficult. This would also constitute a problem for adult fish undergoing return migrations to freshwater after spawning. Loss of riparian vegetation, de-snagging, declining water quality, and changes to hydrology are also probable threatening processes that have contributed to reduced numbers (Hutchison *et al*. 2002; Pusey *et al*. 2004).

Since the 1990s fishways have been installed on many barriers in east coastal catchments of Queensland (e.g. Stuart & Mallen-Cooper 1999; Berghuis 2001; Stuart & Berghuis 2002; Berghuis 2003; Marsden *et al*. 2003a,b). In the last decade various natural resource management groups, catchment groups and local governments have been actively involved in riparian zone restoration. For example, there has been considerable riparian zone restoration work in the Mooloolah River catchment by Waterwatch and Landcare, local government and landholders (Jan Kesby, Mooloolah River Waterwatch and Landcare Inc., pers. comm.). Natural recovery of jungle perch populations following removal of barriers to fish movement appears to occur slowly, if at all (Hutchison *et al.* 2002), implying that recolonisation between isolated freshwater drainages via marine habitat may not be common. Modelling by Feutry *et al.* (2013) suggested that southern Queensland populations of jungle perch would be almost totally reliant on localised recruitment and it was suggested there was little prospect of southern populations being colonised by larval drift from the more abundant northern populations. Remaining southern populations are quite small. For example, Henderson (2010) estimated there to be approximately 200 adults in Wyuna Creek, a 2 km long perennial clear water stream on Fraser Island. Eli Creek, the major creek on the eastern side of Fraser Island, probably has a larger number of adults, but it is still not a very large system, being approximately three times the length of Wyuna Creek. Surveys by DAF staff located further jungle perch in other streams on the eastern and western parts of Fraser Island, but the populations all appear to be smaller than those in Wyuna Creek and Eli Creek. The Maroochy River in south-east Queensland also has a small remnant population of jungle perch. The population is so small that locating adult broodstock is extremely difficult. Anglers occasionally capture adult fish and one or two juveniles are reported each year by the South-east Queensland Ecosystem Health Monitoring Program (David Moffatt DSITI pers comm.). The Maroochy River jungle perch population was most likely impacted by the construction of Wappa Dam in the 1960s (Hutchison *et al*. 2002).

There are a number of potential strategies that could be used to re-establish jungle perch populations. One strategy would be to translocate juveniles and adults from extant populations to restored streams within the historical range. Moving adults has the advantage of fish being already well adapted to the wild and therefore likely to have high levels of survival. However taking enough adults to establish

sufficiently genetically diverse populations can have negative impacts on the viability of the donor populations (Todd & Lintermans 2015). Stocking of wild caught juveniles is another option. Todd and Lintermans (2015) estimated that 600 wild juvenile females (and males) would need to be translocated each year for five years to successfully re-establish populations of Macquarie perch *Macquaria australasica* at new sites in the Australian Capital Territory (ACT.) Remnant adult populations of jungle perch in south-east Queensland and the Mackay Whitsunday Region are too few for translocation of wild adults to be a practical solution without impacting on the remnant populations themselves. Efforts to find adequate numbers of juvenile jungle perch for translocation purposes were also unsuccessful.

Feutry *et al*. (2013) suggested restoring streams between the larger viable northern populations and the more southern catchments to enable a gradual recolonisation southward. Stream restoration has merit and this would probably result in some eventual expansion of the range of jungle perch, but this would probably take decades and would require substantial investment. Southern restored catchments are very isolated from the northern catchments, where existing jungle perch populations are large. In addition, the northern populations may not be suitably adapted to southern and central Queensland environmental conditions. For example, there are differences between southern and northern Queensland populations in salinities required for sperm activation (Henderson 2010) and general observations of captive specimens suggest northern fish are less cold tolerant.

The most practical way to re-establish jungle perch populations in the short term would seem to be restocking of suitably restored systems in the Mackay-Whitsunday Region and south-east Queensland with fingerlings produced from locally sourced broodstock. Stream restoration in adjacent catchments to restocked streams could potentially lead to range expansion through recolonisation from nearby restocked areas.

Fish stocking has been an important and successful conservation and management tool in Australia since the late 1970s (Rowland 2013). Species that have been stocked purely for conservation or for both conservation and angling include Mary River cod *Maccullochella mariensis*, eastern cod *M. ikei*, trout cod *M. macquariensis*, Murray cod *M. peelii*, silver perch *Bidyanus bidyanus* and Macquarie perch *Macquaria australasica* (Rowland 2013). Use of stocking as a management and conservation tool in Australia has received some criticism. Rowland (2013) argues that while some criticisms are justified, many have been made on a theoretical basis, using overseas findings with little or no empirical evidence and not taking into account the imperilled status of Australian freshwater fishes in the 1970s and the loss of genetic diversity and biodiversity in the 1900s; the urgent need for rapid action to increase the abundance and distribution of many species and the difficulties and long time frames required to ameliorate environmental degradation.

One of the concerns around restocking includes genetics (Araki & Schmid 2010; Nock *et al.* 2011). Poor hatchery and stocking management can lead to problems. Many hatcheries throughout the world use small numbers of founding fish, which are used to establish hatchery breeding stocks, and then fish are selected from the following generations and used as broodfish (Rowland 2013). This can lead to loss of genetic diversity (Brown 1987). Strategies to overcome this include use of wild broodstock, turnover of broodstock, use of hormone induction techniques to avoid same matings, and stocking progeny from at least five pairs (Rowland 2013). Further rules around hatchery management to avoid genetic and disease issues with stocking are outlined in the New South Wales Hatchery Quality Assurance Program (Rowland & Tully 2004).

Domestication effects are another concern relating to stocking (Hutchison *et al*. 2012a; Brown & Day 2002). Hatchery-reared fish can have poorer foraging and predator avoidance skills than their wild conspecifics (Hutchison *et al*. 2012a; Brown & Day 2002), which can reduce their fitness for survival. Therefore there can be some advantages to stocking translocated wild fish over hatchery-reared fish if sufficient wild fish are available. But frequently this is not possible with threatened species. Deficiencies of hatchery-reared native fish can be ameliorated by pond rearing of juveniles (Rowland, 2013; Hutchison *et al*. 2012a) and pre-release conditioning of fingerlings to live feeds and predators (Hutchison *et al.* 2012a,b)

Stocking of hatchery-reared fish, in conjunction with habitat rehabilitation has been important in the re-establishment of endangered trout cod (*M. macquariensis*) populations (Koehn *et al*. 2013). The importance of a long term approach in the success of these actions was emphasised (Koehn *et al.* 2013). Some early stockings at selected sites led to little success, but a change in the stocking strategy from stocking few fish in small upland waters, to stocking many fish in large waters over the longer term, has led to what are described as self-sustaining populations (Koehn *et al.* 2013).

Prokop (2015) quoted Mark Lintermans as saying "You need to ensure at least some of your stockings encounter favourable environmental conditions. There are good years and bad years in a river and if you only stock for a couple of years, then chances are you may have stocked in two bad years". In a case study of the translocation of the endangered Macquarie perch *M. australasica,* Lintermans (2013) described the ephemeral nature of assessments of success and failure and the importance of long-term monitoring programs. This study showed how prevailing environmental conditions can impact on reintroductions of fish.

With management of breeding pairs in the hatchery, stocking over multiple years can help build the genetic diversity of the stocked population (Rowland & Tully 2004). Conservation stocking programs are not open-ended, but have clearly defined objectives and timetables (Rowland 2013). According to Stuart Rowland (cited in Prokop 2015) conservation stockings are ideally undertaken for three to five consecutive years with the aim of establishing breeding self-maintaining populations. Stocking then ceases and the recovery is monitored.

Most of the fish involved in conservation re-stocking in Australia to date have been fully freshwater species. In contrast, jungle perch are catadromous with a marine larval phase. Success or failure of jungle perch reintroductions will be provided ultimately by evidence of recruitment, but will jungle perch juveniles return to the stream where their parents were re-introduced? There is evidence for some other diadromous species with marine larval phases, of juveniles and larvae being attracted to rivers with suitable habitat or with populations of conspecifics. Whether this mechanism operates in jungle perch remains unknown. The concentration of jungle perch into a few key streams on Fraser Island suggests there may be some water quality or other cues attracting recruits to those streams.

In a preference experiment diadromous *Galaxias maculatus* larvae preferred freshwater over seawater, suggesting that they may respond to reduced salinity around river mouths during settlement. Furthermore, *G. maculatus* preferred water from one river over another river, suggesting active habitat selection may be important in establishing spatial patterns of larvae at settlement (Hale *et al*. 2008). *G. maculatus* were shown to be attracted to the odour of both *G. maculatus* and *G. brevipinnis*, but not to the odours of the unrelated common bully (*Gobiomorhus cotidianus*). *Galaxias brevipinnis* exhibit species-specific attraction, being attracted to the odour of conspecifics but not to the odour of closely related species (Baker & Hicks 2003). In earlier work Baker & Montgomery (2001) showed species-specific attraction of the juveniles of another galaxiid, the banded kokopu (*G. fasciatus*) to adult pheromones.

Work on conspecific odour preferences of the blue gilled bully (*G. hubbsii*) showed attraction to low concentrations of conspecific odour and repulsion to high concentrations of conspecific odour under neutral water conditions. Under naturally odoured water conditions (streamwater) no attraction to conspecific odour occurred at weak odour conditions and only mild avoidance of conspecific odour occurred at high concentrations. This suggests habitat cues were over-riding conspecific cues (Atkinson & Joy 2008). Thus some species are strongly attracted to conspecific odours and others are not. If jungle perch are attracted to conspecific odours, then recruitment back into restocked habitats is likely to be at a higher level than if they are not.

Objectives

The broad objectives were to:

1. successfully release jungle perch fingerlings into suitable south-eastern Queensland and Mackay-Whitsunday regional waterways

2. understand environmental factors influencing post-release survival of jungle perch in rivers.

A more specific objective was to monitor growth and dispersal of stocked jungle perch fingerlings.

Methods

Broodstock collection

Broodstock collection, transport and maintenance are covered in detail in Chapter 1 of this report. To briefly recap, a total of 160 broodstock were held for this project. Broodstock used for this project included fish that had already been held in captivity for several years prior to this project commencing and fish collected more recently in 2012 and 2013. Long-term captive fish included some fish sourced from the Daintree River catchment in far north Queensland. Daintree River fish were used to help develop production techniques but were not used for breeding for the stocking program. The majority of brood fish were collected by backpack and boat-based electrofishing. Broodstock were sourced from Wyuna and Eli Creeks on Fraser Island in south-east Queensland, the O'Connell River north of Proserpine, and from Rollingstone and Crystal Creeks just north of Townsville. Many of the fish were collected as sub-adults or juveniles, in order to have minimal impact on the remnant breeding source populations. These fish were subsequently grown to maturation in captivity. Some additional juvenile fish captured by an aquarium enthusiast from the Brunswick River in northern New South Wales were also donated to the project. Some opportunistic additions to the broodstock have also been made, with juveniles captured from the SEQ mainland. These have not yet been used for breeding and have been held for on-growing to replace ageing broodstock should the reintroduction experiment continue beyond the life of this project.

Fish from the Townsville-Proserpine region were held in separate tanks to fish from Fraser Island and nearby mainland areas. Fish sourced from the Daintree-Cairns region were also held as a separate group. This was to maintain regional genetic integrity for the breeding program. All broodstock were individually PIT tagged so that the identity of fish used for producing fingerlings was known. This will enable management of future production of fingerlings if the reintroduction of jungle perch continues beyond the life of the current project.

Production of fingerlings

Spawning induction and production of fingerlings were covered in detail in the previous two chapters. Fingerlings for reintroduction to the wild were produced in the second and third years of the project. The production methods are summarised here.

Spawning induction

Prior to spawning induction, jungle perch were captured from broodstock tanks following light sedation (AQUI-S at 10 mL 1000 L^{-1}). After capture individual broodstock were placed in an 80 L aerated plastic tub with a dose of AQUI-S at 1 mL 20 L^{-1} for heavy sedation. When fish lost equilibrium they were scanned for a PIT tag to identify the individual. Female fish were checked for egg condition by cannulation. The collected sample was expelled onto a microscope slide and viewed under a compound microscope with the 10x objective. A micrometer was used to estimate egg size. If the majority of eggs were in the size range of 380 μm to 400 μm or larger, then the fish was considered suitable for spawning induction. Suitable fish were measured to fork length and weighed. Female fish were given an injection of Ovaprim solution (Salmon GnRH 20 μg L^{-1} with Domperidone 10 mg mL^{-1})

at the rate of 1 $mL.kg^{-1}$ for most of year two of the project. However, it was found that as fish spent more time in captivity, lower doses at a rate of 0.75 $mL.kg^{-1}$ became effective. In fish larger than 2.5 kg, a dose of 0.5 $mL.kg^{-1}$ was used in year three to reduce risk of fish becoming eggbound. All Ovaprim injections were intra-peritoneal. A 21-gauge needle was used for ease of injection. The injection was made near the base of the pelvic fins, with the needle facing at a shallow angle in a posterior direction to avoid any vital organs. The injection site was swabbed with iodine solution just prior to injection to reduce risk of bacterial infection.

If sedated fish were identified as male they were gently squeezed in the abdominal region to check for running milt. If free running milt or a cohesive milt thread was detected, males were injected with a dose of 1 $mL.kg^{-1}$ Ovaprim solution following the same procedure as for females. For any given spawning four females were induced. Males were induced at a rate of three males for every female. Four spawning tanks were generally used each spawning run. This was done to enable examination of egg size in relation to female size in some production runs and to better manage the genetics of fingerlings produced for stocking.

Following injection of Ovaprim solution, fish were placed in 7000 L spawning tanks to recover from sedation. Spawning tanks were covered with 10 mm mesh netting to prevent fish jumping out. Tanks were well aerated.

Spawning tank set up, egg collection and management

When fish were placed into the spawning tank the salinity (3-5 ppt) and temperature (generally around 26 ºC) were the same as those in the broodstock tanks. Once the full complement of males and females were placed in a spawning tank the water level was dropped to 25% capacity. The spawning tank was then switched to a marine water recirculating system for refilling. Salinity levels in the spawning tank normally rose to full seawater levels over a 12 hour period. If necessary, sea salt was added to the recirculating system to bring salinity levels up to at least 34 ppt. Water in the spawning tanks was heated to 28 ºC with 3000 W immersion heaters and by additional heating in the recirculating system. Each spawning tank had an overflow pipe that ran into a 300 µm mesh egg collecting basket set in a 200 L tub (Figure 1.1). Water was added to the spawning tanks at a rate of approximately 50 $L.min^{-1}$. Overflow water from the tub was returned to the recirculation system. Spawning generally occurred from 48 to 60 h after induction. From around 48 h after induction, egg baskets were checked by staff at 15 min intervals.

Eggs were transferred from collecting baskets to 20 L buckets. The seawater in the bucket was gently swirled and then left to stand for 5-10 min. Dead eggs and debris that collected at the centre of the bottom of each bucket was siphoned out with a 6 mm plastic tube. Eggs were then transferred to 1000 L hatch tanks, which were constructed of fibreglass and covered with a pale blue gel coat. Tanks had a 7º cone bottom. Hatch tanks were supplied with UV treated 1µm filtered seawater. Water was gently flowed constantly into the tanks (<5 $L.min^{-1}$) and flowed out through a central conical screen of 200 µm nylon mesh. Water level was set by an external standpipe system. Tanks were heated to 28 ±0.5 ºC with 600 W immersion heaters. The water was gently aerated by a central air stone to create a wall of bubbles near the conical screen.

After eggs had been in the hatch tank several hours, the air, heaters and water supply were switched off temporarily, the water gently swirled and debris that accumulated in the centre of the base of the tank removed by siphon as before. Following siphoning, air, water and heat supplies were restored.

Between 10 and 14 hours after fertilisation, rafting eggs at the embryo stage were collected from the incubation tanks (see Chapter 1) and transferred to 20 L buckets or 60 L plastic tubs. Buckets and tubs were only filled to 50% capacity. These were then stocked into either 1600 m^2 (3.2 million L) or 225 m^2 (400 000 L) lined ponds. A small proportion of collected embryos were transferred to larval rearing tanks for on-growing for seven to eight days before stocking into ponds (see Chapter 2). Larvae reared in tanks were maintained on copepod (*P. crassirostris*) nauplii and later stages. This is detailed in the previous chapter. Rafting yolk sac larvae were also collected from incubation tanks (by gently

skimming from the surface with jugs) and stocked into ponds. Any larvae that could not be collected as rafting larvae were harvested by dropping the level of their incubation tank by draining through a 200 µm conical screen set in the centre of the tank. The tank was drained to approximately 10% capacity, to concentrate the remaining larvae. These were collected by scooping water from the tank with plastic jugs and beakers and gently transferring the water and larvae to buckets or plastic tubs for transport to ponds.

Pond rearing of larvae and fry

Embryos and larvae were transferred to the ponds by floating the tubs and buckets of larvae in the pond. Pond water was added to the buckets and tubs, until the tubs and buckets contained 50% pond water and 50% incubation tank water. Larvae were acclimated for approximately 10 min then gently released from the tubs and buckets into the pond. Ponds that successfully produced fingerlings were stocked at a density of less than 0.5 larvae and embryos per L. These ponds also had with a minimum copepod nauplii density of 130 nauplii.L^{-1} at time of first feed and a minimum salinity of 32 ppt. These ponds also had sustained blooms of copepod nauplii above 50 naupii.mL^{-1} until at least 20 days post hatch of larvae. Ponds were gently aerated and plankton blooms were managed by a fertilisation regime and use of blue dye as described in Chapter 2. If adult copepod densities declined below 50 per L by day 20 after hatch, supplementary feeding of larvae in the pond was done by addition of *Artemia* nauplii (see Chapter 2 for details). Weaning diets were also supplied to ponds from 20 days post hatch. Weaning diets included barramundi dust, O. Range small weaning diet and the NRD weaning diet range. Initial feeding rates were a twice-daily broadcast feed of 50 g of mixed weaning diets and 50 g of mixed weaning diets loaded onto an automated belt feeder. Feed rates were gradually increased as larvae grew.

It is likely post metamorphosed larvae (from 28-34 days post hatch) fed on various invertebrates in the pond, including chironomid larvae in addition to the weaning diets. When jungle perch fry all exceeded 25 mm TL (50 to 60 days post hatch), they were captured by drain harvesting into a shade cloth mesh trap set in a pit (see Chapter 2 for details), then transferred to an 800 L capacity fish transporter containing oxygenated, UV-treated filtered seawater.

Indoor rearing of fingerlings

Tank set up and changeover to freshwater

Newly harvested fingerlings were transferred from the fish transporter to rearing tanks. Rearing tanks were 7 000 L or 10 000 L in volume and initially filled with filtered UV treated seawater. These tanks were run on a flow-through system (20 L.min^{-1}). Heaters in the tanks maintained water temperatures at approximately 25 ºC. Tanks were covered with 5 mm mesh to prevent fingerlings from jumping out and outlet drains were screened with coarse shade cloth or 3 mm oyster mesh. Jungle perch fingerlings were held in seawater for just 48 hours. During this phase any post-harvest mortalities were removed and larvae were fed the same weaning diets as provided during pond rearing.

At the end of the 48 h saltwater tank rearing phase, the seawater inflow was shut off and freshwater was run into the tank at a rate of approximately 10 L.min^{-1}. The freshwater inlet was a 25 mm hose weighted to submerge the outlet, which was also screened with 3 mm mesh to prevent fry from attempting to swim up the hose. At this stage newly harvested fry have an extremely strong instinct to migrate to freshwater and they are strongly attracted to the freshwater inflow. If the inflow is not submerged, the fry will jump at the inlet. Submerging the inlet and covering the tank with 5 mm mesh netting are both critical to prevent loss of fry. They are extremely adept jumpers and can easily jump 50 cm or more above the water level.

Tanks eventually became completely freshwater. Initially (for two weeks) fingerling rearing tanks were run as flow-through systems for quarantine purposes. Eventually the fingerling tanks were connected to a recirculating biofiltration system (see Chapter 1 for details) at a flow rate of

approximately 25 L min^{-1}. The water in the recirculating system was maintained at a salinity of 3-5 ppt to reduce risk of parasites and diseases such as whitespot.

Fingerlings were held for varying periods of time. Those harvested late in the spawning season (May) of 2013/14 were held over winter for release in spring. Those harvested during the summer of the 2014/15 spawning season were only held for six to eight weeks and released in autumn in SEQ or early winter in the Mackay region.

Feeding

Jungle perch fingerlings readily take commercial pellet feeds. For the first month, fingerlings were fed to satiation three times per day then after that, twice per day. Initially we used a mix of Ridley's barramundi crumble and 400-800 μm NRD starting diet. The size of pellet feeds were increased as fingerlings grew. Fingerling diets were occasionally supplemented with live invertebrate feeds such as small mealworm larvae and opportunistically collected mosquito larvae from static freshwater containers on site. The proportion of live invertebrate components in the diet was increased two weeks prior to stocking as part of pre-release conditioning.

Predator recognition training

Two weeks prior to stocking fingerlings were conditioned to predators. Approximately 25% of fingerlings to be stocked were conditioned. Work by Hutchison *et al*. (2012b) suggested that schooling species acquire predator conditioning quite rapidly from social learning, cued by behaviour of their conditioned conspecifics. As jungle perch juveniles tend to school, it was believed that conditioning just a subset of all the fingerlings to be stocked should help reduce predation across the whole group through social cues, such as release of alarm pheromones. Fingerlings were conditioned using a modified version of the conditioning method used by Hutchison *et al*. (2012b). A 4 mm oyster mesh screen set in a Perspex frame was fixed across the centre of a 7000 L volume tank. An aluminium plank was run across the top of the screen to create an overhang to hinder fish from jumping over the screen. After inserting the screen the tank was filled to volume of 3500 L. Cover, including PVC pipes and shade-cloth habitats were placed on one side of the screen (predator side) and shade cloth habitat only was placed on the other side of the screen. Predators likely to be encountered at release sites were introduced to the predator side of the screen. To represent southern release site predators, an Australian bass, spangled perch and long-finned eels were used. To represent Mackay–Whitsunday release site predators, a mangrove jack, long-finned eel, spangled perch and barramundi were used. All predators used were small adults or sub-adults large enough to prey upon jungle perch fingerlings. Up to several hundred jungle perch fingerlings were released on the other side of the screen to the predators.

Fingerlings were able to see and smell predators on the other side of the screen. To condition fingerlings to predators, jungle perch skin extract, prepared according to the methods of Ferrari & Chivers (2006) was introduced to the predator side of the tank three times per day (9 am, midday and 3 pm) for three days. Skin extract contains alarm pheromones. It was hoped that associating jungle perch alarm cues with the site and smell of potential predators would condition exposed fingerlings to recognising potential predatory threats. A similar method has been used successfully for other native Australian freshwater fish species (Hutchison *et al.* 2012b). During the conditioning period, fingerlings were fed pellets twice per day to satiation and predators were fed whitebait or prawns once per day.

Micro-tagging

One week prior to stocking, jungle perch were micro-tagged. The first batch of fingerlings released were tagged with Northwest Marine Technology (NMT) wire batch tags. Prior to tagging fingerlings were sedated with AQUI-S. When fingerlings were fully sedated, a small magnetic wire tag (approximately 1.1 mm in length and 0.2 mm in width) was injected with a NMT coded wire tagging machine just beneath the skin in either the right rear dorsal or right front dorsal area. After tagging,

fingerlings were placed in a chute with gently flowing freshwater that transported them to a 300 L recovery tank. After recovery fingerlings were dip-netted and returned to their rearing tanks.

The different body locations for the tags were used as batch marks to denote different release locations. The tags could be detected by running an NMT electronic wand just above the surface of the fish. The wand beeps and a red light flashes when a tag is detected. The wand is able to distinguish different tag locations.

All other fingerlings released were marked with NMT fluorescent visible implant elastomer (VIE) tags. Fingerlings were tagged in the right rear dorsal area just beneath the skin using an NMT air-driven tagging machine. Red and green fluorescent tags were used as batch marks to denote different release locations. Pre-tagging sedation and post-tagging recovery procedures were the same as for wire-tagged fish.

VIE tags fluoresce under blue or UV light and become even more visually enhanced when viewed through amber-coloured glasses. Specially designed blue light torches can be used in underwater surveys to detect tagged fish, although this is usually more effective at night. Research suggests that VIE tags have no significant effect on predation rates of fingerlings (Haines & Modde 1996; Malone *et al*. 1999; Roberts & Kilpatrick 2004; Reeves & Buckmeier 2009; Bouska & Paukert 2010).

Stocking

Site selection

Sites chosen for reintroduction of jungle perch were within the species' historical range. Three small coastal catchments were selected. Two were located in south-eastern Queensland (Currumbin Creek and Mooloolah River), and one just north of Mackay (St Helens Creek). The selected catchments all had what was believed to be suitable habitat for jungle perch. All three catchments had forested headwaters in either state forest or national park with rainforest vegetation. Downstream of the headwaters there were substantial areas with intact or restored riparian vegetation, although some sections still had agricultural disturbance, especially in the lower sections of the catchments. Artificial barriers to migration were either insubstantial (drowning out frequently in the case of Currumbin Creek) or had been removed (Mooloolah River) or rectified with rock ramp fishways (St Helens Creek). The presence of catadromous, and amphidromous species in all catchments suggested that upstream and downstream migration was no longer a critical issue in any of the selected catchments. Water clarity in all three systems was generally good (>1.5 m) and the water was exceptionally clear in St Helens Creek. Observations by the project team of remaining wild jungle perch populations in other stream systems suggested to us a preference for clear water.

Figure 3.1: A jungle perch release site in Currumbin Creek.

Pre-release surveys

Between two and three release sites were selected in each catchment. Figure 3.1 shows one of the release sites in Currumbin Creek. At each of these sites pre-release surveys were conducted to determine what other fish species were present and to confirm the absence of jungle perch. This helped determine likely predators and prey species at each site and provided baseline information. Surveys were conducted by boat or back pack electrofishing. Backpack electrofishing used a Smith-Root Model-12B-POW Backpack Electrofisher operating a 500-volt Pulsed-DC current and a standard pulse setting (1 ms) was used in shallow waters or where travel by foot was required. An operator and single dip-netter were employed during backpack electrofishing activities, with the dip-netter standing downstream of the operator and collecting fish washed downstream with the current.

In St Helens Creek boat electrofishing was done in deeper water with a 3.7 m electrofishing vessel (Hypnos II) which operated a Smith-Root 2.55 GPP electrofisher unit, two boom arms with four dropper anode arrays and a cathode hull. Electrofishing was conducted through each pool, sampling all habitats and areas of the pool. An operator and single dip-netter were employed during boat electrofishing activities. In Currumbin Creek boat electrofishing was used at a single site. A 4.1 m vessel (Small Fry) with a 5.0 GPP electrofisher unit was used. In all other respects the boat set up and operation were similar to Hypnos II.

Where feasible, additional sites were also sampled by the same methods or visual observations were made to confirm the absence of jungle perch. During warm weather jungle perch can be easily observed through polaroid sunglasses, simply by tossing a pebble into a stream. If jungle perch are present they will often rush to the site of the splash to investigate. The flag markings on the tails of jungle perch make them readily identifiable from other species.

General habitat characteristics of each of the release sites were also recorded, including proportion of undercut bank, maximum water depth, proportion of pool and riffle, bottom substrate, area of snags, aquatic macrophytes and emergent vegetation, overhanging vegetation and canopy cover in a 50 m length of stream. Samples of aquatic macroinvertebrates were also collected to determine food availability.

Transport and release

Jungle perch were transported in an insulated fibreglass fish carrier with two 400 L compartments. If there were more than one group of batch-tagged fish being released then each batch was held in a separate compartment. Compartments were filled with 1 μm filtered water, with a salinity of 3 ppt. The water was sourced from the recirculating aquaculture system in which jungle perch fingerlings were reared at BIRC. During transport the water was oxygenated through fine bubblers and supplementary aeration was provided by an air pump that gently bubbled air through air-stones. Fingerlings were transported from BIRC to the south-east Queensland release sites within two hours. Fish transported to St Helens Creek were driven to the release site within 13 hours. A team of drivers rotated driving to enable almost non-stop transport to the release site. Fingerling condition, water pH, oxygenation and aeration were checked every two hours during driver change overs.

A small batch (42) of larger fingerlings (>100 mm FL) was transported to Mackay by air in plastic bags inflated with oxygen and containing 25% by volume 3 ppt water. Bags were sealed with rubber bands and packed into foam boxes. There were four or five fish in each bag. The bagged and boxed fish were collected from Mackay airport then driven to the release sites at St Helens Creek.

Table 3.1: Release locations, release date, number of fingerlings released, transport method, mean release size and tag type used for jungle perch stocked into south-east Queensland and the Mackay-Whitsunday region.

Release site	Date	Number of fingerlings and transport method	Mean fork length mm	Tag type
Currumbin Creek	10 Nov 2014	900 (road)	92.8	Wire
St Helens Creek	27 Nov 2014	42 (air)	150.0	VIE
Mooloolah River	16 April 2015	864 (road)	51.4	VIE
St Helens Creek	21 Jun 2015	1500 (road)	47.5	VIE

On arrival at a release site fingerlings were acclimated to the receiving waters. Bagged fish were floated in bags at the release site for approximately five minutes (Figure 3.2). Water from the release site was then added to the water in the bags and after a short time bags were partially submerged and fingerlings allowed to swim out into the receiving waters. Fish transported by road were dip-netted from the fish transporter and transferred to half full 20 L buckets. Buckets were part filled using the transporter water. To aid dip-netting of fingerlings, the fish transporters were partially drained through screened outlets. Buckets of fish were taken to the receiving waters and partially submerged to allow mixing of the transport water and the receiving waters. After a few minutes fingerlings were released from the buckets into the receiving waters (Figure 3.3). Water quality parameters, including oxygen, salinity, pH, temperature and Secchi depth were recorded at each of the release sites during each release.

Figure 3.2: Releasing bagged fingerlings into a site (Johansen Road) on St Helens Creek.

Figure 3.3: Releasing jungle perch into Currumbin Creek (left) and a school of just released jungle perch fingerlings (right) in Currumbin Creek.

Post-stocking surveys

Post release surveys to locate jungle perch fingerlings were conducted in all three release catchments at least two weeks after release, then at intervals of two to three months after release. Surveys were conducted at locations both upstream and downstream of the release sites. The survey methods varied according to the stream width, depth and accessibility. At areas where there was adequate depth, width and access, boat electrofishing was used. Sites that were shallow enough to wade and not too remote to carry in back pack electrofishing gear were sampled by backpack electrofishing. Electrofishing was conducted as described for pre-stocking surveys. Some post-stocking sites had no boat access or were too deep to sample safely by backpack electrofishing. These sites were sampled by a combination of bankside polaroid surveys (viewing into the stream with the aid of polaroid sunglasses to cut surface glare) and snorkelling surveys. At St Helens Creek, only upper catchment locations were sampled by snorkelling, as crocodile risk had to be considered in the lower reaches. Bankside polaroid surveys were used where it was considered unsafe to enter the water. Tossing of pebbles or pieces of stick into the water aided detection of jungle perch by polaroid surveys. Sections of stream sampled were 50 m to 100 m in length.

In the upper part of St Helens Creek, underwater video surveys were used in conjunction with snorkelling surveys because the water was so clear. A dual video rig was deployed into various locations within a pool, suspended 500 mm above the bottom and left for 10 min to record fish that passed the camera. The dual video rig consisted to two 170^{o} field of view 1080p video cameras affixed back to back to cover a 340^{o} arc. Video from each sampling pass was reviewed on computer, with any fish passing the lens recorded to species level if it could be identified. Where a clear identification could not be recorded, the fish was not included in the data. A blue light torch was also used in conjunction with the video rig to detect VIE tags.

All surveys recorded the presence of the different fish species captured or observed. Numbers of jungle perch observed or captured were recorded and estimates of size made. If jungle perch were captured, they were measured to fork length and were checked for the presence of VIE tags (with a blue light torch) or wire tags (with an electronic wand). The blue light torch was also used in underwater surveys to confirm the presence of VIE tags. Fish in Currumbin Creek were tagged with wire tags. Only captured fish could be scanned with the tag detecting wand, therefore it was not possible to confirm the presence of tags in fish in Currumbin Creek detected through visual surveys. General habitat characteristics, as described for the pre-release surveys were also recorded at the survey sites.

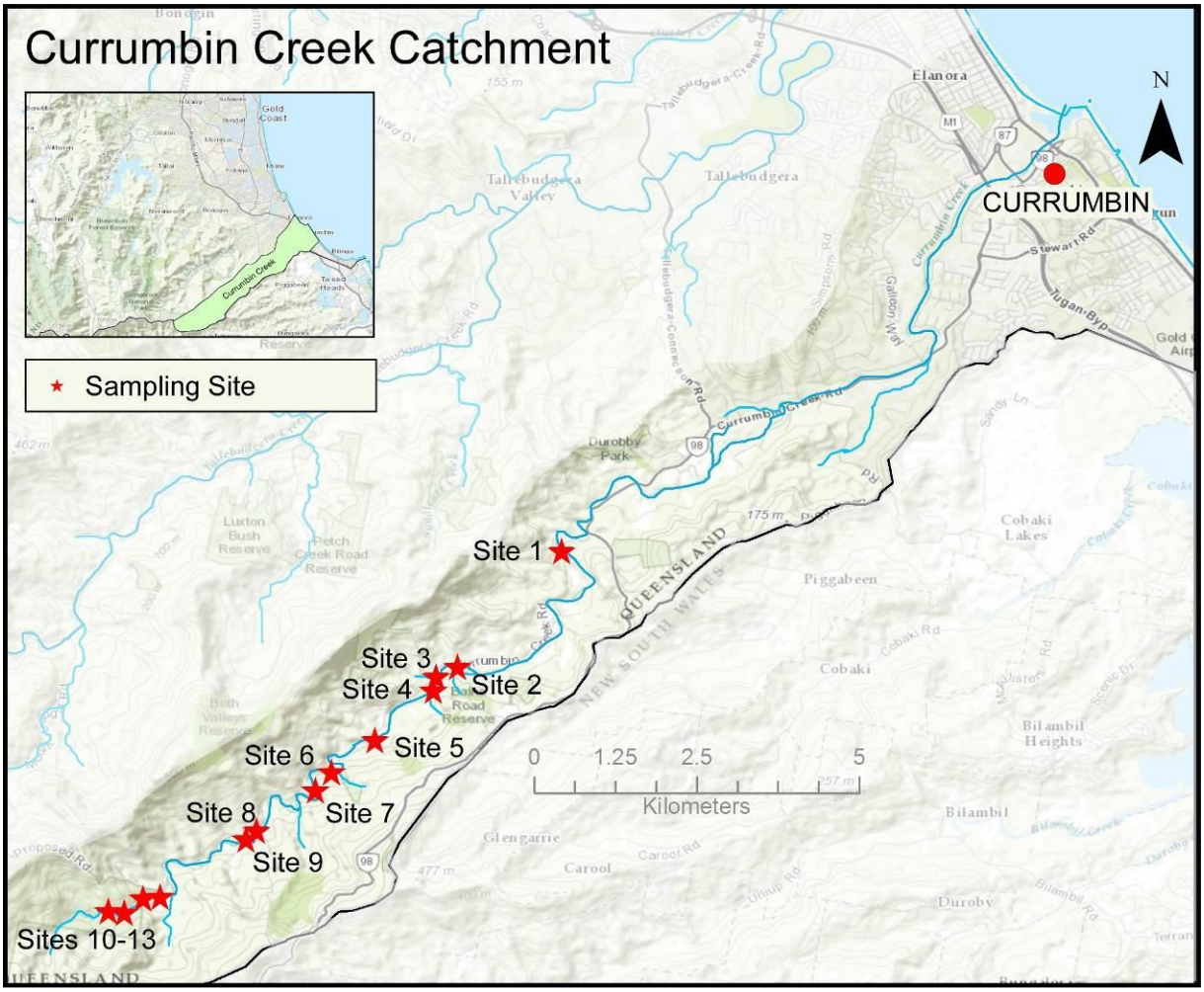

Figure 3.4: Post-stocking survey sites in the Currumbin Creek catchment. Sites 2, 4, and 8 were also used as release sites for fingerlings.

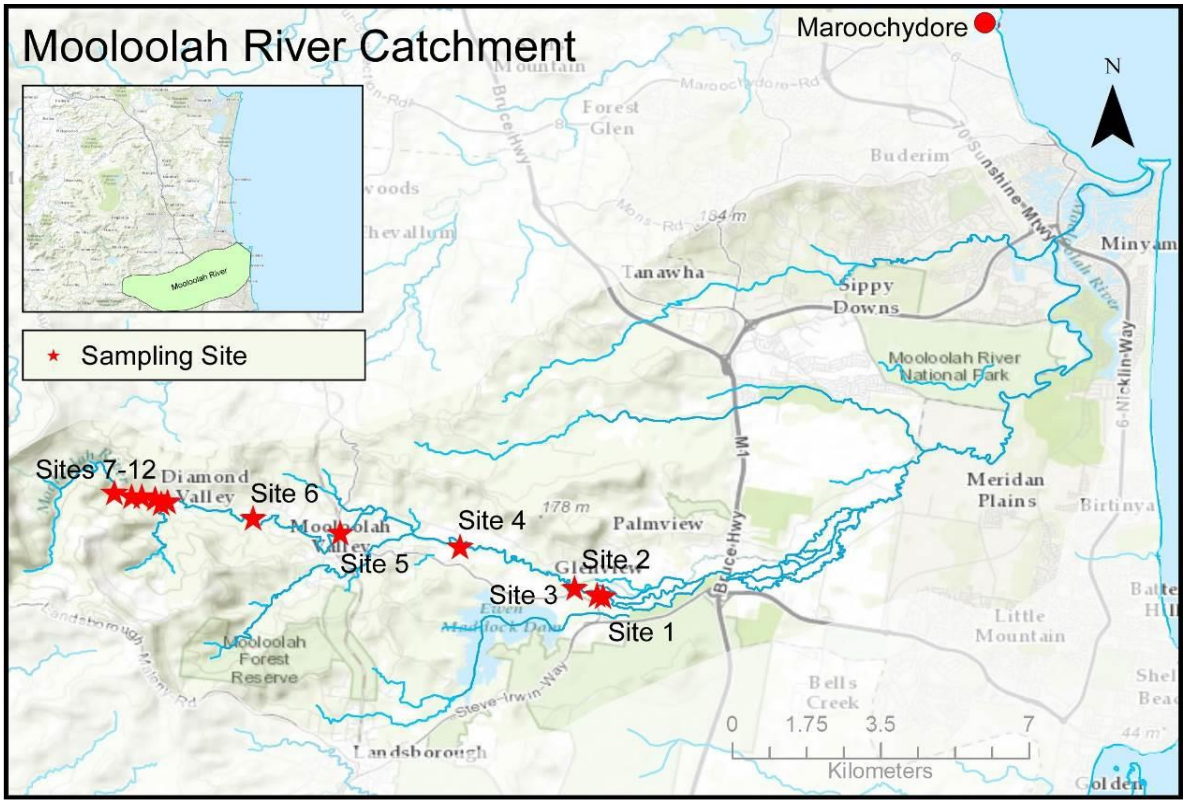

Figure 3.5: Post stocking survey sites in the Mooloolah River catchment. Sites 2 and 8 were also used as release sites for fingerlings.

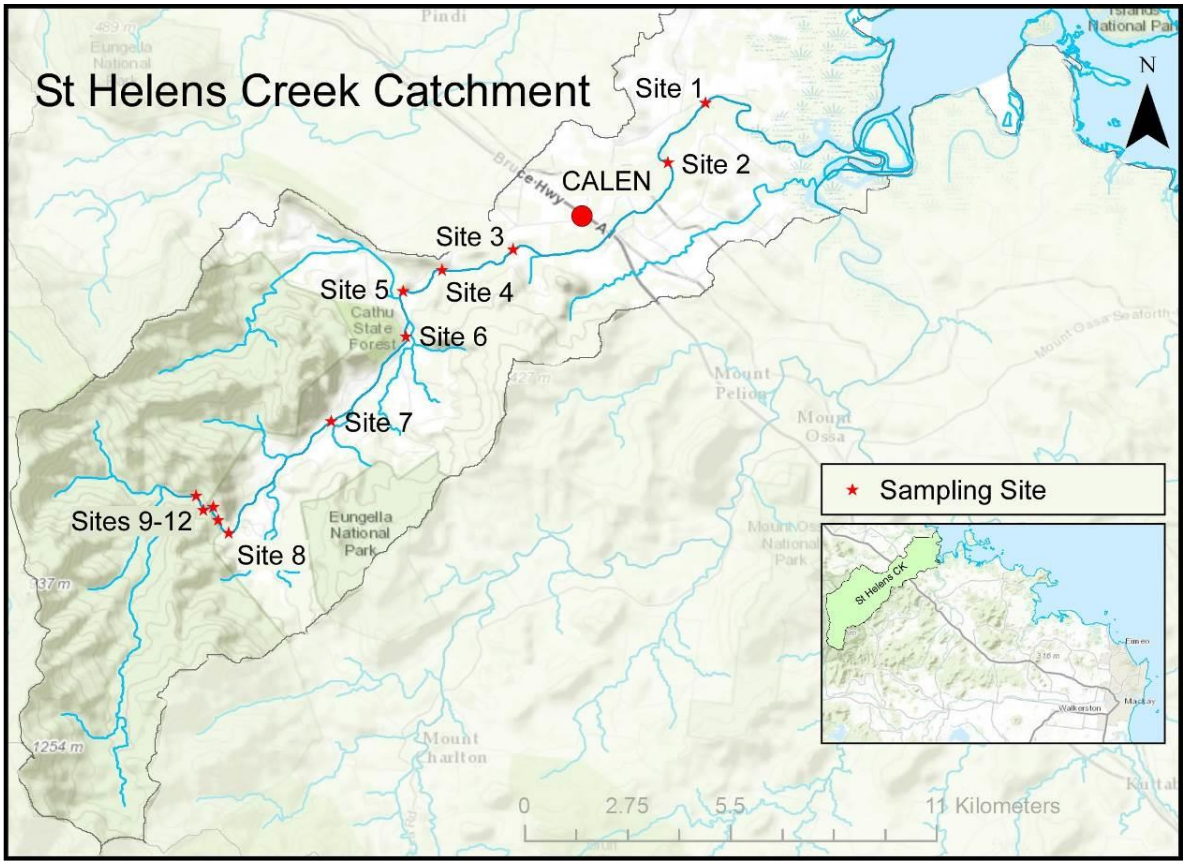

Figure 3.6: Post stocking survey sites in St Helens Creek. Sites 3 and 8 were used to release large fingerlings in Nov 2014 and site 6 to release small fingerlings in June 2015.

Large proportions of all three catchments could not be accessed for surveys of any kind. Lack of road access and lack of access across private properties were the main reasons for this. Post-stocking surveys were therefore a series of point surveys. Figures 3.4, 3.5 and 3.6 show sites that were sampled in the Currumbin Creek, Mooloolah River and St Helens Creek catchments respectively. Release sites are also indicated in the same figures.

Odour preference study

Surplus fingerlings were used to test for odour preference of juvenile jungle perch. Jungle perch fingerlings used in the experiment were approximately 100 mm FL. This was a larger size than preferred but the experiment was run only after all fingerlings required for stocking had been released. Odour preference was tested in a preference trough (fluvarium) with two choices of water provided (Figure 3.7). The preference trough was constructed of Perspex. The upper part of the preference trough consisted of two parallel divided upper chambers 40 cm deep, 120 cm long by 26 cm wide. The lower 20 cm of each upper chamber was filled with a stack of drinking straws, weighted down under plywood boards with a brick. A foam block was also placed above the straws to prevent fish from jumping over the straws. The straws directed a laminar flow into a lower chamber, which was 120 cm long by 52 cm wide and 40 cm deep. The lower end of the bottom chamber has a 70 mm high lip to set the depth of the water in the chamber. A 4 mm plastic mesh screen was fixed 20 cm forward of the lip to allow water to flow out, and to prevent escape of fish. This created a 100 cm section into which fish could be introduced. Freshwater was run through an activated carbon filter and directed to two 1000 L tanks. One of these tanks also contained two small adult (250-300 mm FL) jungle perch, the other tank contained freshwater only. Water was pumped from each of these tanks and directed into one each of the upper chambers at a rate of 10 $L.min^{-1}$. Flow rates into the chambers were adjusted by outlet taps. T pieces directed excess water to waste.. Prior to running any experiments, vegetable dye was added to the inlet chambers and run into the lower chamber to confirm separation of the two water sources in the lower chamber. Experiments were run in a controlled-temperature room, where the air temperature was set at 26 ± 0.5 ºC. Water temperatures in the room remained close to 25.5 ºC for the duration of the experiment.

Ten jungle perch fingerlings were stocked into the lower chamber and left for 2 h. A clear Perspex cover was placed above the chamber to prevent jungle perch from jumping out. Small gaps were provided under the cover to prevent condensation and to obscure vision of the fingerlings. A video camera positioned above the preference trough was used to record the location of jungle perch fingerlings in the tank at the end of the two-hour period. The camera was activated wirelessly and the image viewed remotely on a smart phone. Numbers of fingerlings on the jungle perch odour side of the chamber were recorded for each replicate group. This process was repeated with ten replicate groups of ten fingerlings. The side of the chamber receiving blank water or water with jungle perch odour was alternated to eliminate side-of-tank effects. Between alternate treatments the preference trough was flushed with blank water through both chambers to remove any residual jungle perch odours.

A generalised linear model of binomial proportions with logit link function was run in GenStat ™ (version 16.1) where the proportion of fingerlings using the jungle perch odour side of the trough was calculated. The predict function was used to calculate back-transformed mean proportions of habitat use and standard error of the mean (SEM) values. The null hypothesis of no significant difference from a predicted 0.5 (equal use of either side of the trough) was tested using a two-tailed t test. The value of t was calculated using the formula (0.5-$\bar{x}$)/SEM. Probability levels were calculated using nine degrees of freedom.

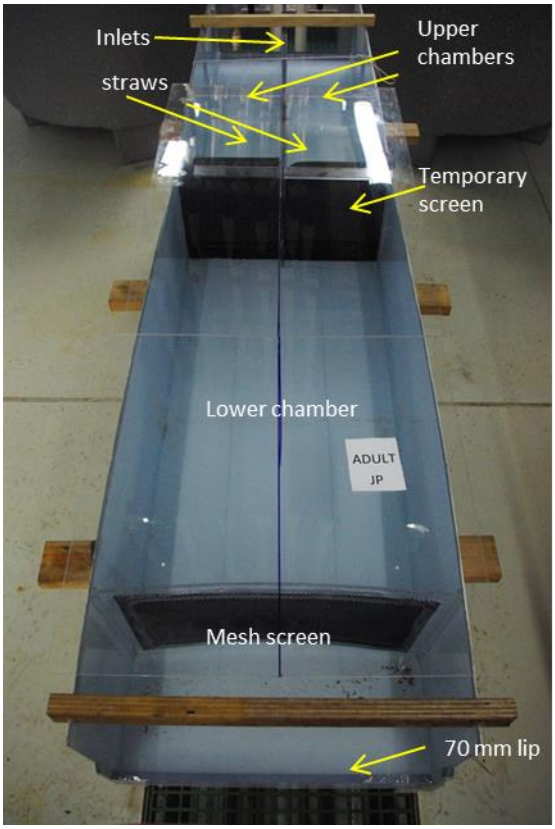

Figure 3.7: Preference trough (fluvarium) showing separated upper chambers with water inlets, a block of drinking straws (behind a temporary screen) to create laminar flow, lower observation chamber with a 4 mm mesh screen set 20 cm above a 70 mm lip for maintaining water levels. Source tanks are in the background.

Results

Pre-release surveys

Prior to the stocking of jungle perch into the three catchments, no jungle perch were recorded from any of the sites sampled. None were observed in general observations around each of the catchments. No jungle perch had been recorded in Ecosystem Health Monitoring Program sampling in either the Mooloolah or Currumbin systems. The fish communities of the five sites pre-sampled in St Helens Creek were consistent with previous sampling conducted in the catchment by Tim Marsden, with a total of 17 species observed during sampling from all sites. A mixture of freshwater resident and diadromous species were captured (Table 3.2). The most common species recorded pre-stocking from all sites in St Helens Creek were empire gudgeons and fly-specked hardyheads.

Pre-release surveys at two sites in Currumbin Creek and at two sites in the Mooloolah River contained typical fish fauna for small south-east coastal river systems, and included both freshwater resident and diadromous species. Seven species were recorded in pre-release surveys in Currumbin Creek and 10 species in pre-release surveys in the Mooloolah River. The fish species captured or observed in pre-release surveys in Currumbin Creek and the Mooloolah River are listed in Tables 3.3 and 3.4 respectively. Ornate rainbowfish and Australian smelts were among the more common species recorded.

Pre-release surveys also found aquatic crustaceans (aytiid shrimps and macrobrachium prawns) to be abundant at the release sites. Aquatic insect fauna were also abundant. Food did not appear to be a limiting factor for jungle perch. Physicochemical parameters recorded were all within the acceptable range for jungle perch (Pusey *et al*. 2004).

Post-stocking surveys

After the stocking of jungle perch in the St Helens catchment, jungle perch were recorded from only one of the 12 sites in St Helens Creek: site 12. Site 12 is the most upstream large pool in the system, located well into Eungella National Park. Jungle perch were detected at three sites in Currumbin Creek (sites 3, 6 and 8) and at three sites in the Mooloolah River (sites 3, 9 and 10) after stocking.

A total of twelve sites were sampled in St Helens Creek post stocking, with some sites visited multiple times. In total, 21 species were recorded from the 12 sites (Table 3.2). Long-finned eels and barramundi were two of the more widespread predatory species recorded. Thirteen sites were sampled in Currumbin Creek post stocking and twelve sites were sampled in the Mooloolah River post stocking. In addition to jungle perch, 15 species of native fish were detected in Currumbin Creek and 16 species of native fish were detected in the Mooloolah River, and two non-native species (swordtail and sailfin molly) were also detected in the Mooloolah River. Non-native species were absent from the majority of sites. All species captured or observed in the south-east coastal systems are listed in Tables 3.3 and 3.4. Smelts and ornate rainbowfish were among the most common species in both south-east coastal systems and long-finned eels were the most abundant predatory species. Gudgeons were the most species diverse group present. Generally, more species were detected where electrofishing could be used, but snorkelling surveys were also effective at identifying multiple species.

All sites that were resampled recorded similar fish communities to the pre-stocking sampling, with some sites recording some additional species (Tables 3.2, 3.3 and 3.4).

Jungle Perch

In St Helens Creek, jungle perch were only observed from site 12 post-stocking (Table 3.2) and did not occur at any other site during this sampling. At site 12, jungle perch were recorded in two distinct size classes, those around 60-100 mm long (Figure 3.8) and those from around 180-250 mm (Figure 3.9). The sizes for both groups are an approximation made from underwater video, snorkelling and visual observation of the fish. In total, 30 individuals of the smaller size class and eight of the larger size class were recorded from the observations.

It was observed that immediately after the larger fish were released in November 2014 at site 8 in St Helens Creek, that they schooled together and began moving in an upstream direction. The fish were tracked several hundred metres upstream until they were lost from sight in one of the upstream pools. These fish were then observed from site 12, several months later, during the post-stocking sampling. It is highly likely that they are the same fish as no fish were observed in the pool prior to the stocking and the group of fish were of the appropriate size for the time they had been released. The smaller fish that were stocked in June 2015 have also moved from the waterhole where they were stocked (site 6). An extensive electrofishing survey of the release pool one week after the stocking occurred failed to identify any jungle perch, despite several hundred other fish being observed. These were also later found to be located in site 12, with a group of 30+ individuals observed during snorkelling surveys. These fish were also positively identified as stocked fish, as no juvenile and sub-adult jungle perch were observed prior to the stocking and the underwater video in concert with a blue light demonstrated that the fish were tagged.

Another observation of the jungle perch within site 12 was the variation in growth rate within the two size classes of fish. It was observed that there were distinctly larger fish in both groups. In the larger sized stocked group from November 2014, there were fish that were approaching 250 mm in size, while at the same time there were a number of fish that were not much greater than their release size of 150 mm. Also in the smaller sized stocking group from June 2015, there were many fish approaching the 100 mm size, while at the same time there were a number of fish still only around the 60 mm size. This size variation (60-100 mm) was also observed in fingerlings from the June 2015 batch held back in tanks at BIRC over the same time period.

The smaller-sized stocked fish were also able to negotiate large natural barriers in their movement to site 12. As there were no major flow events since their stocking, these fish must have ascended three large cascades to reach the upstream habitat. One of these cascades is over 4 m high (Figure 3.10), while the other two barriers were smaller, but with greater vertical jumps.

Figure 3.8: 80-100 mm length jungle perch at Site 12 St Helens Creek

Figure 3.9: Larger jungle perch (approximately 200 mm) at Site 12 St Helens Creek.

Figure 3.10: A significant natural barrier that was ascended by juvenile jungle perch to reach Site 12 St Helens Creek.

Figure 3.11: Jungle perch approximately 100 mm in length one month after release in Site 8 Currumbin Creek.

Post-stocking surveys failed to detect any jungle perch at the two lower release sites in Currumbin Creek, but six fish were found by visual survey within the most upstream release site (site 8) one month after stocking (Figure 3.11, Table 3.3). These fish were estimated to range between 90 and 120 mm in length. A further specimen was captured from an undercut bank at site 8 by an Ecosystem Health Monitoring Program (EHMP) team from DSITI in April 2015, five months after release. This

specimen was retained by EHMP. It had grown to 140 mm FL. Scanning with a wand confirmed it was a stocked fish.

Other jungle perch fingerlings were observed one month post release at site 3, the Currumbin Rock Pools (Table 3.3). This was approximately 200 metres upstream of the most downstream release site, site 2. Three fish were observed here by snorkelling and were estimated to be between 100 and 110 mm in length. Another nine fish were located by visual survey at site 6 (Table 3.3), more than 2 km upstream of release site 4 and over 2 km downstream from release site 8. These fish were all estimated to be between 100 mm and 110 mm in length.

No jungle perch were observed or captured at any site in Currumbin Creek after early May 2015. In early May a major flood occurred in the Currumbin Valley, with waters peaking at 6 m above base flow levels. Most natural barriers to migration would have drowned out in this event, including several waterfalls 4 to 5 m high in the upper catchment.

In the Mooloolah River, jungle perch fingerlings were recaptured at three sites (Table 3.4). All recaptured jungle perch were found to have VIE marks. Only one recaptured jungle perch was stocked at the lower release site. It was recaptured several hundred metres upstream from the release site at site 3. All other recaptures were of fingerlings released at site 8. These were recaptured between 200 and 500 metres upstream at sites 9 and 10. Those recaptured at site 10 had successfully passed upstream of a 900 mm high waterfall, during base flows. Eleven fish were recaptured from sites 9 and 10. Recaptured jungle perch in the Mooloolah River ranged from 38 to 70 mm fork length. Recaptured fish had only been at large for two weeks. One week later in early May a major flood affected the Mooloolah River valley. Floodwaters were up to six metres above normal river levels. Most natural barriers would have completely drowned out during this event. As for the Currumbin Creek, no further jungle perch were observed or recaptured during surveys after the flood event.

Habitats where jungle perch were recaptured included both pools and runs. The fingerlings were predominantly observed in pools, sometimes at the head of the pool near inflowing cascades or runs, but also at the tail of pools. All habitats with recaptured jungle perch had good cover, including rocks and boulders, undercut banks, macrophytes or emergent vegetation. Site 3 on the Mooloolah River was not rocky, but sandy. It still had good instream cover in the form of logs, emergent vegetation and undercut banks. Jungle perch occupy similar habitats on Fraser Island. All sites with jungle perch recaptures had riparian shading. No fingerlings were found in very shallow habitat (less than 40 cm depth). Figure 3.12 shows the preferred habitat of fingerlings in St Helens Creek.

Figure 3.12: Preferred habitat of juvenile jungle perch in St Helens Creek.

Table 3.2: Pre and post stocking sampling results from St Helens Creek indicating presence (X) and absence (blank) of species. Methods include, BE - Boat Electrofishing, BP – Backpack Electrofishing, UV – Underwater Video, OBS – Observation, SS – Snorkelling Sampling.

Site No.	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10		Site 11		Site 12	
Methods Used	BE		BE		BE, BP		BE		UV		BE, BP		BE		OBS		OBS, UV, SS		OBS		UV, OBS		OBS, SS ,UV	
Pre or Post Stocking	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Jungle Perch																								X
Banded Grunter				X	X	X		X				X		X	X	X								
Fly-specked Hardyhead		X	X	X	X	X		X				X		X	X	X				X	X	X	X	X
Mouth Almighty			X	X	X	X		X				X		X										
Empire Gudgeon		X	X	X	X	X		X		X		X		X	X	X	X	X		X	X	X	X	X
Spangled Perch								X				X		X	X	X		X		X			X	X
Eastern Rainbowfish		X	X	X	X	X		X				X		X	X	X	X	X		X	X	X	X	X
Purple-spotted Gudgeon						X																		
Hyrtl's Tandan				X																				
Bony Bream		X	X	X						X		X			X	X								
Eel-tail Catfish						X		X				X		X	X	X	X	X					X	X
Long-finned Eel		X	X	X	X	X		X				X		X	X	X								X
Barramundi		X	X	X		X		X						X										
Bullrout		X	X	X				X						X										
Forked-tail Catfish														X										
Mangrove Jack			X																					
Tarpon		X	X	X	X	X																		
Sea Mullet		X	X	X				X																
Swamp Eel		X		X																				
Roman Nose Goby					X																			
Pacific Blue-eye												X			X	X		X						
Threadfin Silver Biddy		X																						
Mosquitofish			X	X								X												

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Table 3.3: Pre and post stocking sampling results from Currumbin Creek indicating presence (X) and absence (blank) of species. Methods include, BE - Boat Electrofishing, BP – Backpack Electrofishing, UV – Underwater Video, OBS – Observation, SS – Snorkelling Sampling.

Site No	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10		Site 11		Site 12		Site 13	
Methods used	OBS		BE BP OBS		BP SS OBS		BP OBS		BP OBS		SS OBS		OBS		BP OBS		BP OBS		BP SS OBS		SS OBS		SS OBS		SS OBS	
Pre or post stocking	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Jungle perch						X						X				X										
Australian bass				X						X																
Empire gudgeon			X	X																						
Carp gudgeon spp			X	X		X						X			X	X										X
Firetail gudgeon																										
Striped gudegon			X	X		X									X	X				X		X		X		X
Cox's gudgeon								X				X								X		X				
Flathead gudgeon				X		X																				
Dwarf flath. gudegon				X		X																				
Duboulay's rainbowfish												X														
Ornate rainbowfish						X	X	X		X		X		X	X	X		X		X		X				
Long-finned eel			X	X		X	X	X		X		X		X	X	X		X		X				X		X
Eel tailed catfish			X	X		X	X	X		X		X		X	X	X		X								
Australian smelt	X	X	X			X	X	X		X		X		X	X	X		X		X		X				
Sea mullet	X	X	X	X		X	X	X				X				X										
Freshwater mullet				X								X														

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Table 3.4: Pre and post stocking sampling results from the Mooloolah River indicating presence (X) and absence (blank) of species. Methods include, BE - Boat Electrofishing, BP – Backpack Electrofishing, UV – Underwater Video, OBS – Observation, SS – Snorkelling Sampling.

Site No	Site 1		Site 2		Site 3		Site 4		Site 5		Site 6		Site 7		Site 8		Site 9		Site 10		Site 11		Site 12	
Methods used	BP		BP		BP		BP OBS		BP OBS		BP OBS		OBS SS		BP OBS		BP OBS		BP OBS		BP OBS		BP OBS	
Pre or post stocking	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Jungle perch						X												X		X				
Australian bass																		X		X				
Spangled perch												X		X	X					X		X		X
Empire gudgeon		X	X	X		X				X		X			X	X		X		X		X		
Carp gudgeon spp		X	X	X		X						X		X	X	X		X		X				X
Striped gudegon		X	X	X				X		X		X			X	X		X		X		X		X
Cox's gudgeon																				X				
Flathead gudgeon		X	X	X		X									X									
Dwarf flath. gudegon				X		X														X				
Duboulay's rainbowfish												X		X	X			X		X				X
Ornate rainbowfish		X	X	X		X		X						X	X	X				X		X		
Long-finned eel		X	X	X		X		X		X		X			X	X		X		X		X		X
Eel tailed catfish				X								X		X	X	X		X						
Australian smelt						X		X		X		X		X	X	X		X		X		X		X
Sea mullet						X								X										
Freshwater mullet						X																		
Bullrout				X		X												X						
Swordtail												X												
Sailfin molly												X												

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Preference test

There was no significant preference by jungle perch juveniles for the jungle perch odour side of the fluvarium over the blank water side of the fluvarium. Mean use of the jungle perch odour side of the fluvarium was 0.57 ± 0.062. Although the mean use of the jungle perch odour side was more than 50%, this was not significantly different to the null hypothesis level of 0.5 at nine degrees of freedom ($t = 1.129$ $P > 0.05$).

Discussion

For stocked fish most mortalities occur immediately after stocking, in the first few hours or days, rather than weeks (Olla *et al*. 1994; Brown & Laland 2001; Sparrevohn & Støtrupp 2007). Much of this loss is attributed to predation. We know that some jungle perch fingerlings survived this critical period because individuals were observed or recaptured weeks and months after initial stockings. However, it is likely that some jungle perch, especially the smaller size groups, may have fallen victim to predators such as long-finned eels, other carnivorous fish species and to piscivorous birds. The fact that 8 out of 42 fish stocked in St Helens Creek at a size of approximately 150 mm were relocated, suggests that survival of this larger size class was particularly high. Higher survival of larger size classes of several other native Australian fish species has also been noted in experiments by Hutchison *et al.* (2006).

Generally jungle perch did not remain for long in their release locations (site 8 in Currumbin Creek excepted). The high densities of jungle perch at release sites would have necessitated them spreading out to prevent localised overexploitation of food sources. Recaptures were generally upstream of release points, although fish from site 6 in Currumbin Creek could have originated from either site 8 (upstream of site 6) or from sites 2 and 4 downstream. However, observations of fish immediately after release in St Helens Creek, and observations of captive jungle perch fingerlings at BIRC swimming into currents, suggests most of the early movements were most likely upstream.

For two separate stockings of fish from three separate stocking locations to end up in the same waterhole in the upper reaches of St Helens Creek is most interesting, and unlikely to be random behaviour. Does this represent attraction to suitable habitat types, or were the latter group of fish stocked attracted to the earlier group of stocked fish? The smaller fingerlings had moved approximately 6 km upstream from their release point and the larger fingerlings had moved at least 1 km upstream and perhaps as much as 10 km if they originated from the most downstream release site. Apart from a single fingerling recaptured at site 3 on the Mooloolah River, all other recaptures and observations were of groups of fish. This suggests juvenile jungle perch tend to cluster in groups.

The clumping behaviour of jungle perch has made relocating the stocked fish difficult, as they tend to group together within specific locations within the area they are found. This has been observed in St Helens Creek, Mooloolah River and Currumbin Creek (current project) and by project staff in the O'Connell River and in rivers to the north. In effect, when in low numbers they may occur in one section of one waterhole within an entire river system, so locating that one spot can be problematic.

The fluvarium trial suggests that attraction to conspecific odour may not be a factor. However, the batch of fish used in the fluvarium trial were tested at a size larger than the second batch of jungle perch stocked into St Helens Creek in June 2015. Further fluvarium testing with small fingerlings should be trialled. Fingerlings just harvested and ready for changeover to freshwater from seawater may be the ideal stage for this type of testing. If small jungle perch fingerlings are attracted to the odour of conspecifics, it would most likely be at the stage when they are first migrating into freshwater from marine waters that such behaviour would be of advantage. Streams with existing populations of jungle perch are likely to have suitable habitat. Therefore, jungle perch odour could be a useful cue to guide juvenile fish into suitable streams and habitats. If this attraction were to exist, establishment of stocked jungle perch in coastal river systems could potentially attract new recruits back to those same rivers.

Recaptures from the current project suggest juvenile jungle perch preferred the upper sections of the catchment. However, there were many sections of lower, middle and upper catchment that were not accessible, so this information is not conclusive.

Major flooding in both the Mooloolah River and Currumbin Creek appears to have had a negative impact on recaptures of released jungle perch. There are a number of possibilities why this may have occurred. The flood may have displaced fingerlings downstream or into off-stream wetlands we could not access, or did not survey. Alternatively the flooding may have facilitated movement into upper and middle sections of the catchments (including some tributaries) that we could not access or did not survey, or the flood may have had a negative impact on the released fingerlings. In the Mooloolah River, a number of pools where we might have expected to locate jungle perch fingerlings prior to the flood were completely changed after the flood. Large amounts of sand were deposited in some pools, making them much shallower. Over time moderate flows will probably re-scour these pools, but there has been a temporary loss of suitable instream habitat in some sections.

The major flood event reinforces the concept of needing to stock a system over several years to determine if reintroduction is a success or failure (Lintermans 2013; Prokop 2015). Continued stocking over at least another three to five years would also be required to build up adequate genetic diversity in the released populations (Rowland 2013; Prokop 2015). Modelling by Todd and Lintermans (2015) suggested translocation of Macquarie perch was unlikely to be successful unless 600 female young of the year or 100 age 1+ females were released annually for five years.

Numbers of jungle perch released were not very high for the sizes of the stream systems stocked. In the Mulgrave River, natural densities of jungle perch average 1.6 fish per 100 m^2 or 160 $fish.ha^{-1}$(Pusey *et al*. 2004). In some habitats, such as short rainforest streams in the Cape Tribulation area, jungle perch can be very abundant (Pusey *et al*. 2004). Based on the population size estimate for Wyuna Creek (Henderson 2010) and knowledge of the length and width of this stream, densities of adult jungle perch in this system are approximately 5 adult fish per 100 m^2. Numbers of juveniles in Wyuna Creek were not estimated, but observations by project team members suggest they are at least as abundant as adults.

Based on the above population densities, it would appear feasible to increase the number of fingerlings released into the three trial reintroduction stream systems. Some post-release predation and mortalities from other causes are to be expected (even with pre-release conditioning). Therefore, higher numbers of fingerlings probably need to be released into each trial stream in the future (at least several thousand per annum) to establish a reasonable base population. With increased numbers of jungle perch in the system and as stocked fish mature into adults, they may spread into more locations within the catchment and become easier to detect and monitor. In more densely populated jungle perch streams north of Townsville and in far north Queensland, jungle perch seem to occur in good numbers in both lower and upper sections of some streams, penetrating as far upstream as the first impassable high waterfall. Pusey *et al.* (2004) report jungle perch historically occurring hundreds of kilometres upstream in Burdekin system prior to construction of weirs.

Locating jungle perch fingerlings in a 6-to-12 month period after stocking does not prove the reintroduction is a success. It merely proves some survival and growth of fingerlings. Fish recaptured after at least one month at large all showed evidence of growth. The apparent dimorphic growth observed in fish in St Helens Creek parallels the growth observed for fingerlings retained in tanks at BIRC. We believe this may represent sexually dimorphic growth. Mature males definitely grow more slowly than mature females, but at what point sexually dimorphic growth rates diverge is yet to be established.

Monitoring of restocked systems ideally should continue over several years. If possible, an expanded number of monitoring sites should be established. This will require negotiated access to additional private properties in the lower and middle reaches of the trial systems or hiking into more remote upper catchment sites. If markers were ever developed for jungle perch, e-DNA could be used as another monitoring tool. Proof of success would be a demonstration of natural recruitment into the restocked systems or into adjacent restored systems. It will take two to three years for the first batch of stocked fish to reach maturity. Migration of new recruits into the stream systems is likely to occur between January and May. Setting of fine-mesh fykes or

use of electrofishing or night time spotlighting surveys around small natural barriers in the lower sections of these streams are the most likely methods to detect new recruits.

The current EHMP program already detects small numbers of juvenile jungle perch in the Maroochy River each season (David Moffatt DSITI pers. comm.). The Maroochy is one system in south-east Queensland with a small remnant population of adult jungle perch. Detection of new recruits in the restocked systems therefore appears feasible.

If natural recruitment into restocked streams is detected, then potentially reintroduction of jungle perch fingerlings could be expanded into other restored stream systems. Stocking larger numbers of fingerlings than have been released to date is more likely to lead to success.

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Conclusion

Development of hatchery production techniques for jungle perch fingerlings

This project has developed hatchery production techniques that have led to the production of jungle perch fingerlings for the first time. To optimise egg quality, broodstock diets should include predominantly aquatic or marine components. Terrestrial dietary components such as giant mealworms may contribute to growth of jungle perch broodstock, but are probably not important for production of high quality eggs. However, giant mealworms are a favoured food of jungle perch broodstock, and may have some value in gauging broodstock health.

Spawning of broodstock and incubation of eggs require salinities of at least 32 ppt for the best results. Temperatures in the range of 27-28 ºC appear suitable for spawning, egg incubation and larval rearing. Copepod nauplii are an essential first feed for jungle perch larvae. Feeding cannot be achieved with rotifers or oyster trochophores. First feeding takes place three days after hatch. Copepod nauplii densities in excess of 1 mL^{-1} seem to be required to provide larvae with adequate nutrition to penetrate the surface tension and inflate their swim-bladders. Swim-bladder inflation occurs between four and seven days after hatch. Lighting of at least 2500 lux appears essential to promote feeding and survival. Jungle perch larvae do not cope well with upwelling or turbulent water in a tank. Gentle aeration appears to be essential for rearing success.

For most hatcheries, long-term production of sufficient quantities of copepods to rear a batch of larvae through to metamorphosis would be logistically difficult. The less labour intensive option is to rear larvae in ponds. Spawning should be delayed until ponds have copepod nauplii densities of at least 130 $nauplii.L^{-1}$. Higher starting densities should produce even more reliable results. After three weeks, pond-reared larvae can be transitioned to *Artemia* and weaning diets. Weaning diets designed for barramundi appear suitable for advanced jungle perch larvae and jungle perch fry.

Larvae metamorphose between 28 and 34 days post hatch. Between 50 and 60 days post hatch, fry are generally large enough and robust enough to harvest from ponds. Harvested fry can be transitioned to freshwater and reared in tanks on diets designed for rearing barramundi fingerlings.

Successful release of jungle perch fingerlings into south-east Queensland and Mackay-Whitsunday regional waterways and understanding environmental factors influencing post release survival of jungle perch in rivers.

This project produced enough fingerlings for trial releases of micro-tagged jungle perch fingerlings into three catchments (Currumbin Creek, the Mooloolah River and St Helens Creek). Some of the fingerlings were recaptured or detected at intervals ranging from a few weeks to 10 months after release. The majority of fingerlings detected had moved upstream from the release locations. Fingerlings tended to cluster in groups and were difficult to detect. They seemed to favour pool habitats with inflowing water and adjacent cover, such as undercut banks, boulders or macrophytes. A large flood affected both the Mooloolah River and Currumbin Creek in May 2015. This flood event negatively impacted detection of jungle perch fry. Whether the flood resulted in mortalities of fingerlings or dispersed them to areas that were unable to be sampled is unknown. It is too early to determine if the reintroductions have been a success. Restocked fingerlings will reach sexual maturity in two to three years. Monitoring should continue beyond three years to detect natural recruitment.

Ideally stocking should continue for another three to five years to build up genetic diversity and critical mass of the restocked populations. Stocking over multiple years also reduces the risk of adverse environmental events negatively impacting the reintroduction of jungle perch populations and will aid the monitoring of

jungle perch populations. The numbers released to date have been quite small and each of the restocked systems could easily cope with higher numbers of released fingerlings.

Communication with anglers on the restoration of jungle perch fisheries

Although not covered in the research chapters of this report, results of this work have been communicated regularly with anglers and hatchery operators, through media releases, posters, e-mails, presentations and displays. These activities are covered in the extension and adoption sections of this report.

Development of a hatchery production manual for fish hatcheries

A hatchery production manual with accompanying video material has been produced as a result of this project and will be distributed to hatcheries in New South Wales and Queensland and to interested international aquaculture organisations. The Hatchery Production Manual is a complementary document to this research report.

This project has resulted in the world's first successful production of jungle perch fingerlings. There are no doubt, further improvements that can be made to the production process. The availability of a manual will enable commercial hatcheries to successfully produce fingerlings. The manual will provide a foundation on which further improvements and refinements to the production of jungle perch can be built. In a period of three years, production of jungle perch has progressed from a point where 100% of larvae died within five days of hatch, to a point where thousands of fingerlings have been produced and a proportion of these used for reintroduction experiments.

Implications

Hatchery Industry

This project has solved the key problems around jungle perch production. The Hatchery Production Manual and other extension activities will enable commercial hatcheries to transition to production of jungle perch fingerlings. At present, jungle perch fingerlings can only be produced for the aquarium industry or for grow-out for human consumption. If jungle perch become a permitted species for stocking, then options for the hatchery industry will increase. Hatcheries will have a new product to offer to impoundment stocking groups.

Restoration of fish stocks

Now that jungle perch fingerlings can be produced, there is an opportunity to properly evaluate whether restocking can re-establish self-sustaining jungle perch populations in restored stream systems. This will require at least three more years of restocking at three pilot sites followed by monitoring for natural recruitment. If southern populations of jungle perch are listed under the *Environment Protection and Biodiversity Conservation Act* (EPBC) as threatened or vulnerable, and the pilot sites have had demonstrated success, then restocking may become part of recovery plans for southern jungle perch populations.

Aquaculture potential

The aim of the current project was not to produce a new species for aquaculture. However, now that production of fingerlings has been solved, there is potential to evaluate jungle perch as an aquaculture species in the future. Preliminary observations suggest that females grow faster than males. Production for aquaculture may need to rely on female stock. Males could be sold to the aquarium trade. Female broodstock on maintenance diets have put on 1 kg body weight in 12 months, therefore probably have the potential to grow fast enough to be an economically viable option for aquaculture. There are various references in the literature to jungle perch being a good table fish. As jungle perch are diadromous they can be reared in freshwater, but are tolerant of saltwater, which could be useful for disease and parasite management and pre-harvest purging.

Stocked Fisheries and Tourism

Jungle perch are an iconic angling species. Should jungle perch become a permitted species for stocking, then they could eventually become part of the impoundment stocking program in east coastal catchments of Queensland. Stocked impoundments are already economically valuable to regional Queensland, with some individual impoundments generating several million dollars per annum for regional economies. The iconic status of jungle perch should help increase that economic value and draw interstate and international tourists to Queensland to target jungle perch. Jungle perch are already targeted by anglers from Japan and Taiwan.

Recommendations

Further improvements to jungle perch production can undoubtedly be made. Areas requiring further investigation or refinement are as follows:

Male contribution to spawning: This project showed some evidence that higher male to female ratios produced better fertilisation rates. This suggests that some males may not contribute adequately to spawning. Research on how to improve male contribution to spawning or improve male spawning condition could lead to further improvements in genetic management of spawns and fertilisation rates.

Further fluvarium trials: Preliminary fluvarium trials found no significant attraction of 100 mm fingerlings to jungle perch odour. Further trials should be conducted with smaller 25-30 mm fingerlings. This is the stage that has just entered freshwater, and migration cues may be more important at that developmental stage. If jungle perch odour is a migration cue, then there is a higher probability of having natural recruitment back into restocked systems.

Transitioning larvae from copepods to rotifers or *Artemia*: For tank rearing of jungle perch larvae to become more economically viable, reducing the period of dependence on copepods is important. Further research needs to be done to determine if jungle perch larvae can eventually be transitioned from copepod nauplii to enriched rotifers, or if they cannot be transferred to rotifers, to determine the earliest possible transition time across to *Artemia* nauplii.

Research on sexually dimorphic growth: Research on growth of jungle perch fingerlings and determining at what stage sexually dimorphic growth commences could be important for the development of jungle perch as an aquaculture species. If males and females could be separated at an early stage, then potentially females could be used for grow-out in aquaculture and males used for the aquarium trade.

Assess aquaculture potential: The potential of jungle perch as an aquaculture species needs to be evaluated. This could be done jointly with the aquaculture industry.

Continue reintroductions at the three test sites: A one-off stocking is insufficient to evaluate the success of reintroductions. Stocking should continue at the three pilot stream reintroduction sites over three to five years to build up genetic diversity and base numbers of fish. After that, stocking should cease and monitoring continue to determine if natural recruitment is occurring. If natural recruitment occurs, then the reintroductions can be considered successful.

Continue a transition period to commercial hatcheries: For commercial hatcheries to take up production of jungle perch fingerlings, there needs to be a transition period where project researchers work with commercial producers as part of ongoing project extension. This will assist hatchery operators to become successful at producing and rearing jungle perch larvae through to fingerlings.

Further development

Nomination of jungle perch to EPBC: As jungle perch numbers have declined significantly over 50% of their Australian range, they would qualify for listing under the EPBC Act. If listed, this would result in development of recovery plans and may expedite the recovery of this species. Listing under the EPBC Act does not necessarily preclude angling. Species such as Murray cod and Mary River cod are also listed, but angling is allowed to continue.

Permitted species: If jungle perch become a permitted species for stocking in east coastal impoundments of Queensland, then more hatcheries are likely to take up production of jungle perch fingerlings. Currently hatcheries are limited to producing jungle perch for the aquarium trade or grow-out for aquaculture. For jungle perch to become a permitted species, this will require amendments to the regulations under the *Queensland Fisheries Act*. There is some logic to jungle perch becoming a permitted species in east coastal

impoundments. Jungle perch are indigenous to east coastal rivers, whereas some of the existing permitted species are not.

Impoundment stocking trial: It would probably be prudent to conduct an impoundment stocking trial of jungle perch under a research permit before making a decision on whether or not jungle perch become a permitted species for stocking. If jungle perch did poorly in an impoundment situation, then there would be little point in it becoming a permitted species.

A number of questions could be answered with a trial in one or more impoundments. (for example, an SEQ impoundment and an impoundment near Mackay).

- Are jungle perch compatible with other stocked species?
- Are jungle perch more readily lost from impoundments compared to other diadromous species (e.g. bass and barramundi)?
- Are jungle perch catchable by anglers in impoundments?
- Are jungle perch fingerlings best released directly into the impoundment or into feeder streams?

Extension and Adoption

Presentations

Presentations were made to the following groups on the following dates:

Aquaculture Association of Queensland (AAQ, the peak body representing fish hatcheries)

AAQ AGM and conference July 2012, August 2013, May 2014

Brisbane Sportfishing Club, September 2012

ANSA, November 2014

Ewen Maddock Fish Stocking Group, June 2013

ANGFA (Aquarium enthusiasts group for Australian and New Guinea fishes) August 2013

Conferences

National Recreational Fishing Conference, Gold Coast August 2012: Poster presentation August 2012

Australian Society for Fish Biology (ASFB) Conference, Sydney October 2015: Paper presentation.

Events

FFSAQ made regular presentations on jungle perch at various FFSAQ events and recreational fishing events including the Tinnie and Tackle Show and the Brisbane Boat Show throughout the course of the project. Posters and video material were made available to FFSAQ. This extension work was primarily done by Mr Les Kowitz.

Australian lure, fly and outdoor expo, July 2015: DAF staff set up a display in a booth in July 2015 outlining the achievements of the project. The display included a video of jungle perch fingerling releases, and videos on production of jungle perch fingerlings and posters on key aspects of the project. An aquarium display of fingerlings was also featured.

Site Visits

ANGFA visited BIRC in early 2014 to see firsthand aspects of jungle perch production.

Pine Rivers Fish Stocking Group toured the BIRC site in October 2013 and were given an overview of the jungle perch project.

A group of recreational fishers from Toowoomba led by Peter Taylor visited the site in May 2014.

Five hatchery operators visited BIRC in early 2015. They were shown some operating stages of jungle perch fingerling production and also shown the general facilities used for producing jungle perch. They were given various tips and information on the production process.

A jungle perch production run has been scheduled for January 2016. This will be used as a demonstration for some interested hatchery operators. Eggs and larvae will be provided to some operators with access to saltwater ponds, or who have some copepod production capacity.

Hatcheries throughout Queensland and NSW were sent a letter in 2014 outlining the proposal to produce a hatchery production manual for jungle perch. In 2016, the manual will be sent to all hatcheries that expressed an interest in receiving a copy.

Project summary brochure

A draft brochure outlining the achievement of the project has been produced. It is intended to provide general information on project outcomes to recreational anglers.

Jungle perch hatchery production manual

A jungle perch production manual with embedded video material has been developed to assist interested hatcheries to produce jungle perch. The hatchery manual will be sent to all interested hatcheries in 2016. A link to the manual will also be posted on the DAF website in early 2016.

Fingerlings to FFSAQ

Jungle perch fingerlings were provided to FFSAQ in July 2015 for use in their displays.

Project web page

A project web page with a link to video material was set up at
http://www.daf.qld.gov.au/fisheries/research/research-projects/developing-jungle-perch-fingerling-production-to-improve-fishing-opportunities

https://drive.google.com/folderview?id=0B0j0c3UCJPPcR0hKSVFfcElENWs&usp=sharing

Project coverage

Videos and stories on Facebook

A video on jungle perch spawning was posted on the Fisheries Queensland Facebook page
https://www.facebook.com/FisheriesQueensland/app_310476315708719

A Facebook story on production of fingerlings is also linked to another video that summarises aspects of the fingerling production.

https://www.facebook.com/notes/fisheries-queensland/more-than-1000-jungle-perch-fingerlings-reared/778882182163420

Press releases

Initial project press release July 2012: “Breeding the next generation of jungle perch.” Appeared in Gympie Times, Queensland Fishing Monthly, Bush and Beach and online at Ausfish, Sweetwater Fishing and The Fish Site.

February 2013: Press release on the first captive produced jungle perch fingerlings: Appeared in local newspapers including the Island Times, Fishing World Magazine web site, and on Fisheries Queensland’s Facebook page and on Twitter.

A general press release on the release of fingerlings was made in late April 2015. This release was published in Queensland Fishing Monthly and Bush and Beach Magazine and also appeared on the Fishing World magazine website. It was also posted on the Fisheries Queensland Facebook page.

Radio interviews

ABC local radio Queensland: Interview on first ever jungle perch fingerlings produced (February 2013).

Television

Scope: Children’s Science program. Covered jungle perch project. Aired Channel 10 October 2012.

FISH Magazine

A story on jungle perch “Jungle perch go wild” appeared in FISH Volume 23 (2) 16-17 June 2015.

Hatchery International Magazine

Hatchery international magazine ran a two-page story on the production of jungle perch fingerlings in 2015.

Project materials developed

Hatchery Manual and Videos: A jungle perch hatchery production manual with embedded videos on various aspects of production has been produced. Given the embedded video material, the manual is available in electronic form only. It will be loaded onto USB drives and posted to various hatcheries. An on-line link to the manual will be established in 2016.

Scientific Paper: A paper linked to this project has been published. A University of Queensland Honours student, Matthew Hoskin, investigated the genetics of progeny produced from spawns involving three females and nine males per tank during the first phase of this project. This resulted in a publication in which the principal investigator was a co-author.

Hoskin, M.L., Hutchison, M.J., Barnes, A.C., Ovenden, J.R. and Pope, L.C. (2015) Parental contribution to progeny during experimental spawning of jungle perch, *Kuhlia rupestris*. *Marine and Freshwater Research* **66**, 375-380.

Abstract ASFB: An abstract of the oral presentation "Bringing back jungle perch" presented at the 2015 ASFB conference in Sydney is published in the Conference book of abstracts.

Poster: A poster on the jungle perch project was produced for the 2012 National recreational fishing Conference in Sydney. This poster was also used by FFSAQ for promotional purposes.

Laminated images

Laminated images of various stages of jungle perch production were produced and used for display purposes at the 2015 Australian Lure, Fly and Outdoor Expo held at the Ipswich Showgrounds.

Draft Brochure

A draft brochure "Developing jungle perch fingerling production" has been produced to summarise the results of the project and to distribute to recreational fishing groups.

Videos

A number of project videos have been produced. Some of these have been linked to a project web page or posted on Facebook.

https://drive.google.com/folderview?id=0B0j0c3UCJPPcR0hKSVFfcElENWs&usp=sharing

https://www.facebook.com/notes/fisheries-queensland/more-than-1000-jungle-perch-fingerlings-reared/778882182163420

Other videos, such as one showing release of fingerlings into the wild, have been used at events such as the 2015 Australian Lure, Fly and Outdoor Expo. FFSAQ have a copy of the Fingerling release video and use it as extension material at their events. Videos on various aspects of fingerling production are embedded in the jungle perch hatchery production manual

Project web page

A project web page was established at http://www.daf.qld.gov.au/fisheries/research/research-projects/developing-jungle-perch-fingerling-production-to-improve-fishing-opportunities

Appendix 1: Project team

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Appendix 2: Intellectual Property

All outputs from this project are intended as public good. The key objectives of the outputs are to restore fisheries and develop the hatchery industry, recreational angling and regional tourism. It is intended that the results of this research be extended and applied as widely as possible for the public good.