Jungle perch *Kuhlia rupestris* fingerling production manual

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Summary

This manual consists of written descriptions of jungle perch *Kuhlia rupestris* production and video material to demonstrate each of the key production steps. Video links are at the end of each major written section in the document. To activate the link use ctrl click. The videos enhance the instructive ability of this manual.

The keys to producing jungle perch are:

- maintaining broodstock in freshwater or low salinity water less than 5 ppt
- spawning fish in full seawater at 28°C
- incubating eggs in full seawater. Salinities must not be less than 32 ppt
- ensuring that first feed jungle perch larvae have an adequate supply of copepod nauplii
- rearing larvae in full seawater under bright light
- use of gentle aeration in tanks
- postponing spawns until adequate densities of copepod nauplii are present in ponds
- sustaining copepod blooms in ponds for at least 20 days
- avoiding use of paddlewheels in ponds
- supplementary feeding with *Artemia salina* and weaning diets from 20 days after hatch
- harvesting of fingerlings or fry after they are 25-30 mm in length (50 to 60 days post hatch)
- covering tanks of fingerlings with 5 mm mesh and submerging freshwater inlets to prevent jumping.

Introduction

This manual is based on the knowledge gained by researchers at the Bribie Island Research Centre (BIRC), working on developing jungle perch *Kuhlia rupestris* captive breeding as part of the FRDC funded project 2012/213 “Developing jungle perch fingerling production to improve fishing opportunities”. Further refinements can certainly be made to improve larval rearing and fingerling production. This manual reports on methods that have worked at BIRC to date, and perhaps more importantly, on what didn’t work. Knowledge of what has failed will help private hatchery operators avoid mistakes as they try to further refine the jungle perch production process in their own facilities.

The manual describes each of the key parts of jungle perch production, including broodstock management, spawning induction, spawning, egg and larvae management, live feed production, pond management, pond harvesting and fingerling management. The manual also includes links to video segments to demonstrate how things were done at BIRC. Videos are integral for the use of this manual. Click on the video link at the end of each production step described in this manual. It is intended that the video segments will enhance understanding of the jungle perch production process. The videos in this document are also available in the attached video folder that accompanies DVD and USB drive versions of this document and can be viewed as stand-alone files. The written document contains the majority of the technical information required, such as stocking densities, fertilising rates, feeding rates *etc*. The videos demonstrate the processes, which words are not always adequate to describe.

The flow chart below shows each of the key steps of jungle perch production described in this manual. Some of the steps are optional, for example rearing of larvae can bypass tanks and rely solely on pond rearing. Live feed production is essential for tank rearing of larvae, but is optional for pond rearing of larvae. Ponds naturally produce live feed, but supplementary production of copepods or brine shrimp can enhance larval production in ponds.

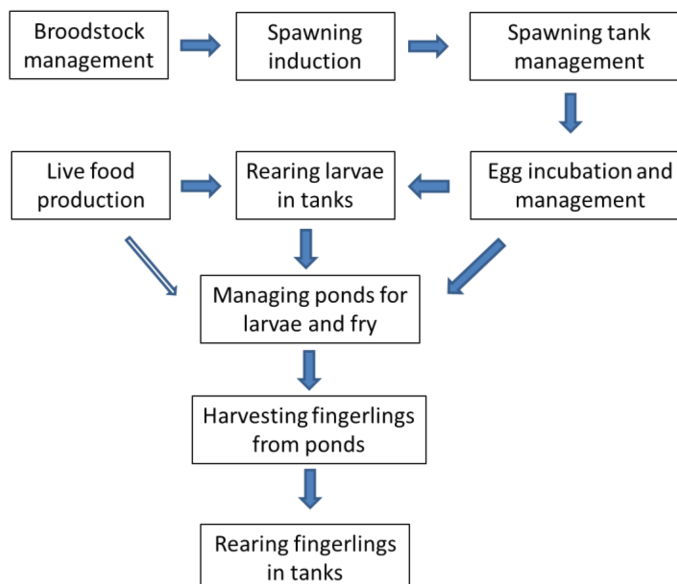


Figure 1: Jungle perch production flow chart. Each step in this flow chart is described in this manual and contains an accompanying video segment.

Link to introductory video

Broodstock management

Tank system

Better control over tank conditions, including water quality, temperature and salinity is possible if broodstock are held in recirculating systems. Broodstock jungle perch at BIRC are held in 7000 L tanks that are well aerated and connected to a recirculating bio-filtration system. The recirculated system includes a 50 micron drum filter to remove solid waste, a closed and exposed bio-filtration system (to remove ammonia and phosphate) with biological towers containing bio-beads with a wagon wheel pattern to increase surface area. The system includes two large variable speed drive pumps. If one pump fails the second pump starts automatically. Water is supplied to broodstock tanks at a rate of 60 L per minute to ensure turnover in each tank every two hours. The recirculation system is linked to a back-up power supply in case of mains power failure. The system also contained programmable heating and chilling systems to regulate temperature, and a UV system to treat the water for bacteria and other parasites. An ozonation system (for water sterilisation) and a foam fractionator (to remove protein) were also part of the system. However these latter components were not used in this project. Foam fractionation is not very efficient in freshwater.

All tanks are aerated to keep oxygen levels high. Conical bottoms help facilitate waste removal. Tanks used for jungle perch broodstock at BIRC have 7° conical bottoms.

Temperature

From October to May water temperatures were maintained close to 26°C (range 23.9°C to 28.2°C) and for the remainder of the year close to 23°C (range 19.4°C - 25.9°C). 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. When necessary, additional submersible heaters can be added to tanks in winter to keep temperatures above 20°C. Broodstock from Fraser Island and further south will feed at cooler temperatures, but jungle perch from further north feed better if the temperature is kept above 20°C. Keeping the temperature above 20°C should also help eliminate any digestive metabolic problems that can occur in some freshwater species at cool temperatures.

Salinity

Jungle perch broodstock should be kept in freshwater or low salinity water. The system at BIRC is maintained at 3ppt to 5ppt salinity. This level of salinity helps prevent white-spot disease *Ichthyophthirius multifiliis* and it also places the fish under less osmotic stress. There have been no disease issues with jungle perch broodstock held at BIRC. Do not maintain broodstock in full seawater. Broodstock tend to feed less well and show signs of stress if maintained in seawater long term. Low salinity or freshwater is best to bring jungle perch into breeding condition.

Stocking densities

Broodstock are held at relatively low densities at BIRC, ranging from 2 kg to 5 kg m^{-3}. That is normally equivalent to 10-12 adult jungle perch in a 7000 L tank. Jungle perch females grow

faster and to a larger maximum size than males. Females can be expected to attain a maximum size of more than 3.5 kg whereas males can be expected to attain a maximum size generally not much more than 1.2 kg.

Net covers

Jungle perch are capable jumpers. They settle in well to captivity, but may jump randomly or to capture an insect near the water surface. Therefore it is important to cover all broodstock tanks with netting. 10 mm or 15 mm mesh should be sufficient to cover broodstock tanks. These also permit feeding of pellets and mealworms by scattering over the mesh. To feed prawns and small fish, small feeding holes can be cut in the net coverings, but it is important these are not too large or fish will jump through them.

Feeding

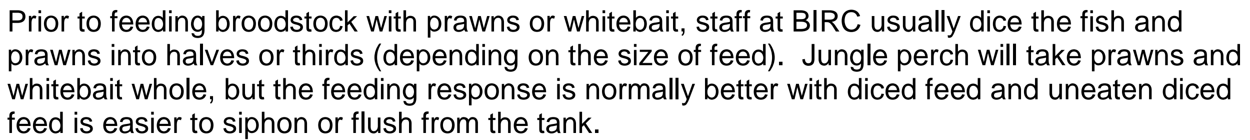
During the southern hemisphere breeding season (including the period from six weeks prior to the breeding season) October to May, jungle perch broodstock can be fed to satiation five days per week. From June to September (southern hemisphere winter and early spring), provided the temperature regime is maintained as described above, meals can be cut back to feeding to satiation just three days per week (Table 1).

In the wild jungle perch consume a range of terrestrial and aquatic insects, along with fish and crustaceans (Pusey *et al.* 2004). Jungle perch broodstock are particularly fond of terrestrial insect components in their diet, including mealworms and giant mealworms. However, a diet containing a greater component of marine or aquatic components was found to be more beneficial to egg quality than a diet dominated by terrestrial components (Hutchison *et al.* 2015).

At BIRC following experimental evaluation of different broodstock diets on egg quality, fish were eventually maintained on a diet consisting of commercial pellets (developed for barramundi), prawns, and small fish (Table 1). Small fish are supplemented by soaking in vitamins every second week. Mealworms or giant mealworms can still be maintained in the diet as a terrestrial component, once per week. Terrestrial insects are probably not essential for a healthy broodstock diet, but they are a useful inclusion. Jungle perch broodstock are particularly fond of mealworms or giant mealworms and will eat them in preference to almost anything else. Feeding mealworms to jungle perch is a useful way to gauge the health of broodstock. If they show no interest in mealworms or giant mealworms, then something could be wrong. There are numerous guides to mealworm and giant mealworm production available on the internet.

Table 1: The recommended feeding plan for Jungle perch broodstock during the breeding season and non-breeding season. * Fish is generally sandy sprats or blue bait.

Day	Breeding season diet	Winter season diet
Monday (week 1)	prawns	barramundi pellets (6mm)
Tuesday	barramundi pellets	
Wednesday	fish*	prawns
Thursday	Mussels or prawns	
Friday	barramundi pellets (6mm)	fish
Monday (week 2)	barramundi pellets (6mm)	barramundi pellets (6mm)
Tuesday	prawns	
Wednesday	fish* +vitamins	prawns
Thursday	barramundi pellets	
Friday	giant mealworms	giant mealworms



Prior to feeding broodstock with prawns or whitebait, staff at BIRC usually dice the fish and prawns into halves or thirds (depending on the size of feed). Jungle perch will take prawns and whitebait whole, but the feeding response is normally better with diced feed and uneaten diced feed is easier to siphon or flush from the tank.

Tank cleaning and flushing

Tanks at BIRC are fitted with a broken siphon system, a 50mm PVC pipe angled from the centre of the tank to a head unit that connects to a drain. The drain can be fitted with mesh if small fish are kept in the tank. As the tank is topped up with recirculating water it rises up the pipe and overflows into the drain (Figures 1 and 2). This system keeps the tank clean by removing most solid waste. The drain flows back to the recirculation system including the drum filter. Tanks are also fitted with a short inner 50 mm central stand pipe covered over with an outer larger taller 90 or 100 mm central stand pipe (Figure 3). The outer stand pipe has a scalloped base. By opening a valve at the base of the tank (Figure 4) water is drawn through the scalloped base of the outer pipe then into the shorter inner stand pipe. This system is also featured in the broodstock management video linked to this section. The inner stand pipe is set to about 25% tank full level and prevents a tank from completely draining. Normally the valve is closed fully after the tank has drained by approximately 10%. This system is used to flush the tank once a week after brushing the bottom to remove any solid waste, or bottom growths in the tank. This water rapidly flows to the recirculating system sump and drum filter. The rate of draw down leads to some overflow in the sump system and results in approximately a 5% water change for the recirculating system. A float valve in the recirculating system will top up any water lost with new freshwater. This weekly water change helps prevent build-up of nitrates in the system and helps to maintain good water quality for the broodstock.

Rather than use the broken siphon system (Figures 1 and 2) it is also possible to run the recirculating system through an inner and outer central standpipe system (Figure 3), but the inner stand pipe will need to be set to the desired normal tank water level. Water changes with this system will need to be more active, by manually running new water into the recirculating system.

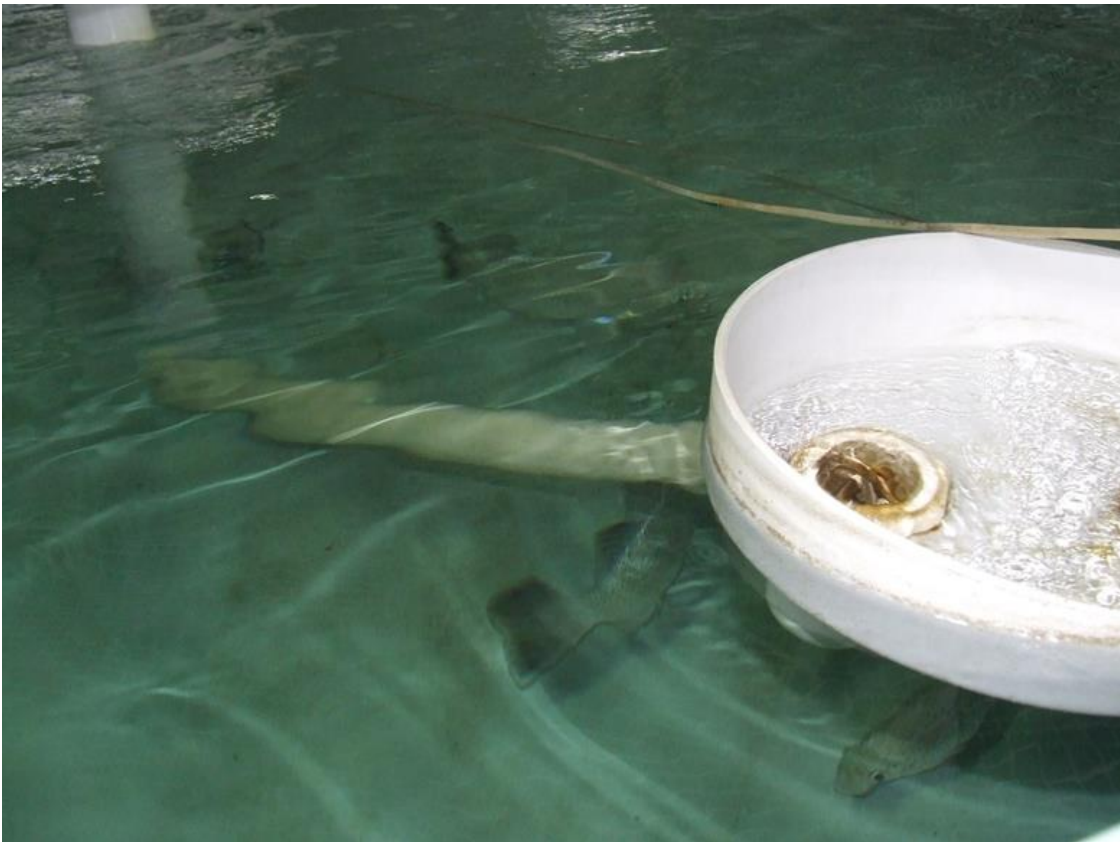


Figure 2: Broken siphon system, with pipe angled toward the centre of the tank. Water runs up to the broken siphon head, and overflows into a drain to the sump of the recirculating system

Figure 3: (left) Head of broken siphon system.

Figure 4: (right) Example of an inner and outer stand pipe. Water is drawn through the scalloped base of the outer stand pipe then up and into the inner stand pipe.

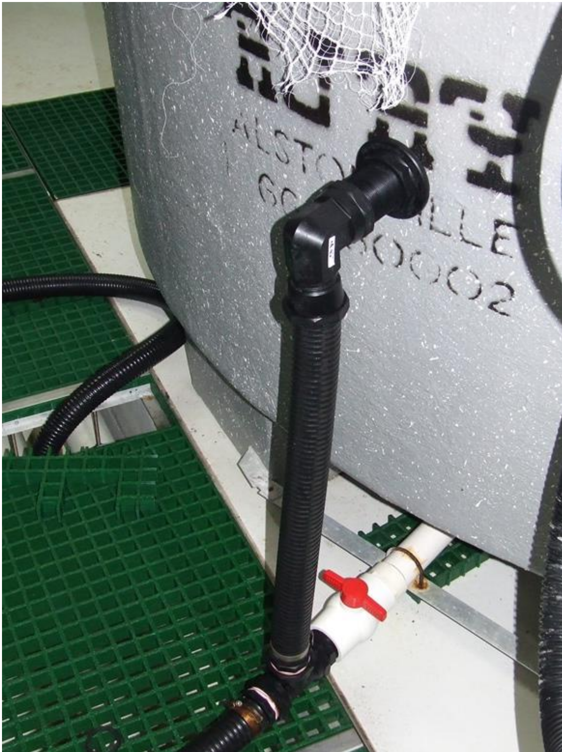


Figure 5: Tank plumbing for overflow from the broken siphon system. If the bottom valve is opened the tank is flushed through the central standpipe system.

Water quality monitoring

The water quality parameters, dissolved oxygen, pH, salinity and temperature are monitored daily with a water quality meter. Adjustments to salinity can be made by addition of filtered seawater or sea salt to keep the tank system running between 3 ppt and 5 ppt salinity. If oxygen levels are lower than 4 ppm, aeration can be increased, or feeding rates reduced temporarily. If pH drops below 6.5, pH can be adjusted up by addition of sodium bicarbonate or a major water change.

It is also advisable to monitor ammonia levels regularly to confirm the bio-filtration system is operating efficiently. If the recirculating system or tank system is topped up with town water, then it is also important to monitor incoming chlorine levels. There are various chlorine and ammonia monitoring kits available from aquaculture and aquarium suppliers. Chlorine can be removed from in-coming water with activated carbon filters or water can be stored in tanks with vigorous aeration for 24 to 48 hours prior to introducing it to broodstock or fingerling tanks.

PIT tags

All broodstock held at BIRC are individually identified with PIT (passive integrated transponder) tags. These are injected into the dorsal muscle region of broodstock. PIT tagging broodstock is useful because it enables hatchery operators to keep track of fish from different regional stocks, monitor individual growth and condition and to manage genetics by identifying which individuals are used in particular spawning events. Once the sex of a fish is known, PIT tags will also speed up the spawning induction process, because it enables identification of the sex of fish on

capture. It is then just a matter of using the appropriate technique to determine the spawning condition of the fish.

Link to broodstock management video

Spawning induction

In Queensland Australia the jungle perch spawning season runs from mid-November through to April (the southern hemisphere late spring through summer to mid-autumn). The spawning season can be extended to the end of May with temperature and photo period manipulation.

Capture and sedation

Broodstock are not fed the day before a spawning induction. This is to minimise faecal contamination of the spawning tank and egg collection baskets. Prior to spawning induction broodstock should be maintained in freshwater or low salinity water (< 5 ppt). On the day of induction the level of tank water should be lowered and AQUI-S or another approved sedative for use in aquaculture can be added at a level for light sedation. For AQUI-S this is a dose of 10 ml per 1000 litres of water. Lightly sedated fish will have less risk of being damaged or stressed during capture, and that will contribute to improved spawning success. Fish should not lose equilibrium at this dose, but they will be easy to dip-net for transfer to a tub for heavy sedation prior to examination for spawning condition. A tub of 60 L containing 2 mL of AQUI-S will provide moderate sedation, and a tub of 60 L containing 4 mL AQUI-S will provide heavy sedation. A two tub system can be used to speed up the examination process. As a fish is transferred from the moderate sedation tub to the heavy sedation tub, a replacement fish from the lightly sedated broodstock tank can be placed in the moderate sedation dose. Heavily sedated fish will lose equilibrium, but still have some gill movement. Be careful not to leave fish under heavy sedation for too long.

Checking reproductive condition

Once a fish is heavily sedated they can be checked for reproductive condition. Female fish can be examined by cannulation. Insert a 2 mm diameter plastic tube (or cannula) into the urogenital opening (the most rear opening) of female fish. The cannula should be sterilised prior to use by washing in alcohol. Insert the cannula slowly until some resistance is felt. Sucking on the far end of the cannula will draw reproductive tissue into the cannula. The contents of the cannula can then be blown out of the tube onto a microscope slide for examination. Oocytes of females in spawning condition will generally be creamy yellow in colour. Less developed oocytes tend to be more translucent. Jungle perch are serial spawners, so normally a range of developmental stages will be apparent in most samples. Fish ready to spawn generally have around 40-50% of oocytes larger than 380 µm in diameter, with some oocytes up to 420 µm in diameter. It is preferable if you have a calibrated micrometre available to insert into the eyepiece of your microscope so oocyte size can be estimated. If a large proportion of eggs are 350-360 µm, but none are over 380 µm in diameter your sample may look ready to the naked eye, but any spawn achieved is likely to be of poor quality. Properly evaluating the size of eggs in your sample can prevent unnecessary waste of hormone.

It is not possible to insert a 2 mm cannula into the urogenital opening of a male jungle perch. If you experience difficulty in inserting a cannula into an unsexed fish don't try to force it, it is most likely your fish is a male. Once the sex of a fish is determined, it can be linked to a PIT tag identification and this can be used to streamline future spawning events and to manage pairings. Males can be checked for spawning condition by applying gentle pressure with

forefinger and thumb, starting at the upper abdomen back down to the urogenital opening (see spawning induction video). Males in spawning condition will have a show of milt. In jungle perch this show usually has the appearance of a white thread, and does not run as in some other fish species. Hormone induction of male jungle perch in this condition will result in successful spawnings.

Male and female fish not in spawning condition should be placed in an aerated freshwater tank to recover from sedation. Once recovered from sedation they can be returned to a broodstock tank. Male and female fish in spawning condition can be prepared for spawning by hormone induction.

Scanning for PIT tags

It is advisable to scan broodstock for PIT tags prior to hormone induction so you can keep track of which fish you have bred from during the spawning season and so you can make decisions as to which males you want to mate with a particular female to maximise genetic diversity over different spawning events.

Hormone injection

Researchers at BIRC have had success in inducing jungle perch to spawn using injectable salmon GnRHa (20µg mL^{-1}) with domperidone (10mg mL^{-1}). This can be purchased as a commercial preparation (Ovaprim). Domperidone is a dopamine antagonist and can enhance the effectiveness of GnRH (Alok *et al*. 1997). Dopamine can block GnRH activity and stress can lead to dopamine release. Other hormones such as human chorionic gonadotropin (HCG) may also successfully induce jungle perch to spawn, but these have not yet been extensively tested on jungle perch.

Fish should be weighed to determine the appropriate hormone dose. The optimal spawning dose for jungle perch is **0.75 mL Ovaprim per kg body weight for females** and **1 mL per kg body weight for males**. A small proportion of large female jungle perch over 2.5 kg body weight can become eggbound with use of Ovaprim, but this has not been seen in smaller fish to date. Reducing the dose for large females (over 2.5 kg bodyweight) to 0.5 mL per kg bodyweight appears to prevent fish from becoming eggbound.

Spawning from very large females can be inconsistent. Some very large females have good quality spawns, but others seem to produce poor quality spawns. Researchers at BIRC generally prefer to spawn from females less than 2.5 kg bodyweight. Once the appropriate hormone dose has been selected, it can be injected into the abdominal cavity just to the rear of the pelvic fin. Swab the injection area with iodine to minimise risk of bacterial infection. Point the syringe needle to the rear of the fish and insert it at a reasonably shallow angle to avoid hitting any vital organs. This is demonstrated in the spawning induction video.

After injection of the hormone dose the jungle perch should be transferred to a spawning tank. Initially the spawning tank should be of the same salinity as the broodstock tank (i.e. freshwater or low salinity).

Link to spawning induction video.

Spawning tank management

Tank size and female to male ratios

Researchers at BIRC spawn fish in 7000 L tanks, with a diameter of 3.1 m. Spawning tanks should be covered with netting as for broodstock tanks to prevent fish from jumping out of the tank. The tank should be well aerated.

A ratio of one female to three males is recommended to maximise chance of fertilisation and to improve genetic diversity of spawns. At BIRC it is usual practice to spawn one female per tank and to use multiple spawning tanks. This enables the identity of females which had successful spawns to be confirmed. It is possible to have successful spawns with multiple females in a tank. For example three females with nine males in a single tank may have successful spawns, but it is not always readily apparent which females have contributed to successful spawns. The only way to determine this is by genetic analysis (Hoskin et al. 2015). Individual female jungle perch have been observed to spawn twice, up to 12 hours apart, so what may be assumed to be two spawns from two different females in a tank containing multiple females, may in fact be two spawns from a single female.

Spawning tank salinity

When fish are placed into a spawning tank the starting salinity should be the same as that in the broodstock tanks, i.e. either freshwater or low salinity water less than 5ppt salinity. When the spawning group have all recovered from sedation, the tank volume can be lowered to around 25-30% full. Filtered seawater should then be introduced to the tank. Allow the tanks to fill and flush at a moderate rate such that they reach full seawater salinity within 12 hours. It is possible to do this on a flow through system, but if the tanks can be put onto a recirculating system with some seawater exchange, it will enable better control of water temperatures and water quality. If you are unable to achieve full seawater salinity due to lack of a sufficient seawater supply, the salinity can be increased by supplementary addition of sea-salt. One kilogram of salt will raise the salinity of one cubic metre of water by 1 ppt. It is important for the salinity of the spawning tank to reach at least 32 ppt and preferable for it to exceed 34 ppt to achieve good fertilisation rates and buoyancy of fertilised eggs.

Spawning tank temperature and timing of spawns

A temperature of 28°C is recommended for spawning tanks. Spawning will still occur at lower temperatures (e.g. 26°C) but the timing is less predictable at lower temperatures. Fertilisation also seems to be more reliable at 28°C. At 28°C spawning occurs at a mean time of 56 hours post induction. The majority of spawns occur between 50 and 60 hours after induction. The earliest recorded spawning time was 36 hours after induction and some spawns have occurred in excess of 80 hours after induction. The very late spawns usually have poor fertilisation rates, but not in every case.

Egg collection

A female jungle perch will normally release between 100,000 and 400,000 eggs per kg of body weight. Fertilised jungle perch eggs are buoyant in seawater. There are two reliable ways the eggs can be collected. The most frequently used system at BIRC is to use an overflow pipe in the tank. The overflow is directed to an egg collection basket in a tub adjacent to the spawning tank. The egg collection basket is made from 250 to 300 µm mesh fitted over a 25 mm PVC pipe frame (see Figure 5) with dimensions of 50 cm x 50 cm x 60 cm deep. The tub has an outlet set lower than the height of the egg basket to prevent the basket from overflowing. The

outlet either runs to a drain (in the case of flow through systems) or is redirected back into the recirculating system. The latter system is used at BIRC to improve temperature control. Alternatively the same style of egg collection basket can be attached to floats and placed in the spawning tank. An air lift, comprising an air-stone in a PVC pipe with an inverted J configuration can be attached to the basket. This will lift eggs from the water column into the egg collection basket. This set up can be used in both flow through and recirculating systems and is also suitable in static tank systems for operators who cannot flow through seawater or who do not have their tank system on a recirculating system. To maintain good water quality it would be preferable to use a recirculating or flow through system for spawning.

Spawning tanks should be well aerated. Aeration aids removal of all eggs from the spawning tank. Even though fertilised eggs are buoyant in full seawater, unfertilised eggs may sink without aeration. Removal of unfertilised eggs is important to maintain water quality in the spawning tank. The unfertilised eggs can be separated from the fertilised eggs after removal from the spawning tank.

Removing eggs from baskets

Spawns are easily detected by shining a torch into the egg collection basket. Allow the eggs to accumulate in the basket for one to two hours before removing them. The easiest way to remove eggs is to partially lift the egg basket out of the water to concentrate the eggs into a smaller volume of water. Wash down the sides of the basket with spawning tank water to get as many eggs as possible into the water at the bottom of the basket. Use a beaker or similar container to scoop out the eggs and water from the bottom of the basket. Tip the contents into a bucket. Researchers at BIRC transfer eggs initially into a 20 L bucket and keep adding eggs and seawater until the volume is exactly 20 L. This is to enable calculation of egg numbers volumetrically.

After removing eggs from the egg basket, the basket can be set back down into the spawning tank or tub and checked again later for further eggs that may come through. The bucket of eggs can be taken to an incubation room for processing.

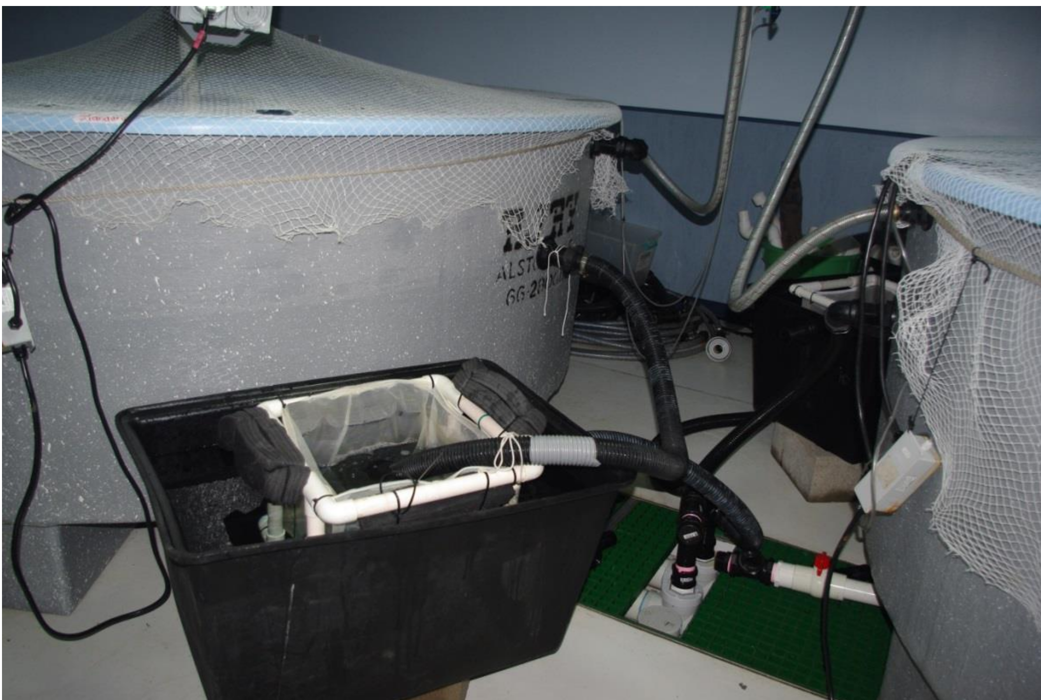


Figure 6: Egg collection basket set in tub to capture eggs from overflow pipe.

When spawning has been completed, broodstock in the spawning tank will need to be returned to freshwater or low salinity water. Broodstock can be changed over to freshwater in the spawning tank. Drain the saltwater level in the spawning tank to around 25% capacity, then commence a moderate in-flow of freshwater to the tank. It is preferable to submerge the freshwater inlet to near the bottom of the tank. For example use a weighted hose. Post–spawning, adult jungle perch are normally highly motivated to return to freshwater. They are strongly attracted to any freshwater inflow. If the inflow comes from the top of the tank and creates splashing on the surface, the fish will jump at the inflowing water. This has the potential to cause injuries to adult fish if they hit the sides of the tank or the inlet pipe. If the incoming flow is submerged, the fish may aggregate near the inflow but jumping behaviour is eliminated. When the tank is fully freshwater it is not necessary to submerge the inflow. It is the urge to leave seawater for freshwater that seems to drive the jumping behaviour.

After the post-spawning adults have been in freshwater or low salinity water for 24 hours, they can be lightly sedated (see capture and sedation in the section on spawning induction), dip-netted and transferred back to their respective broodstock tanks.

Link to spawning tank management video

Egg incubation and management

Estimating egg abundance and fertilisation rates

Gently mix a bucket of eggs by hand to bring all eggs in the bucket into an even suspension. Extract a sample from the bucket with a 10 mL pipette. If the egg sample is of very high density a 1 ml sample should be sufficient. If egg densities look low, extract 5 or 10 ml samples. Drain the sample onto a fine mesh (200 µm) sieve. Place the sample under a dissecting microscope and count the number of eggs in the sample. This will give a number of eggs per ml, per 5 ml or per 10 ml depending on the sample size. Repeat the mixing, sampling and counting five times. Calculate a mean (average) number of eggs per sample. To estimate the total number in 20 litres, multiply the average number of eggs in a 1 ml sample by 20,000. The average number of eggs in a 5 ml sample can be multiplied by 4,000 or the average number in a 10 mL sample can be multiplied by 2,000. For example if the average number of eggs in a 1 mL sample is 21 eggs, then the estimated number of eggs in 20 L = 20,000 x 21 = 420,000.

Fertilisation rates can be estimated by pipetting samples of mixed eggs onto a petri dish. The petri dish can then be examined under the microscope. The number of fertilised eggs (eggs showing cell division) can be counted and expressed as a percentage of the total number of eggs in the sample. This process can be repeated with several samples from the bucket to provide a mean estimate of fertilisation rates.

If fertilisation rates are high, then egg quality is likely to be good and development through to hatch is worthwhile. To maintain water quality in hatch tanks, it is important to separate out dead and unfertilised eggs from the good eggs.

Cleaning up a batch of eggs prior to incubation

Initial removal of unfertilised and dead eggs can be done in the egg collection bucket. It is important for salinities to be at least 32 ppt or higher for this to take place. Fertilised eggs tend

to be buoyant or neutrally buoyant at salinities of 32 ppt or above (Figure 6). Buoyancy increases as salinity increases. If possible it is best to aim for full seawater salinities (34-36 ppt) for spawning and egg incubation. In contrast to fertilised eggs, dead eggs and most unfertilised eggs tend to sink. Gently swirling the water in a bucket of eggs creates a vortex. If a bucket of eggs is left to settle for five to ten minutes after swirling, dead eggs, unfertilised eggs and other detritus tends to collect towards the centre of the base of the bucket. The unfertilised eggs, dead eggs and detritus can then be siphoned (with a 5 mm tube) out of the bottom of the bucket. The majority of eggs remaining in the bucket should be live fertilised eggs. These can then be transferred to an incubation tank. Removal of dead and unfertilised eggs helps prevent a deterioration of water quality and a build-up of bacteria in the incubation tank.

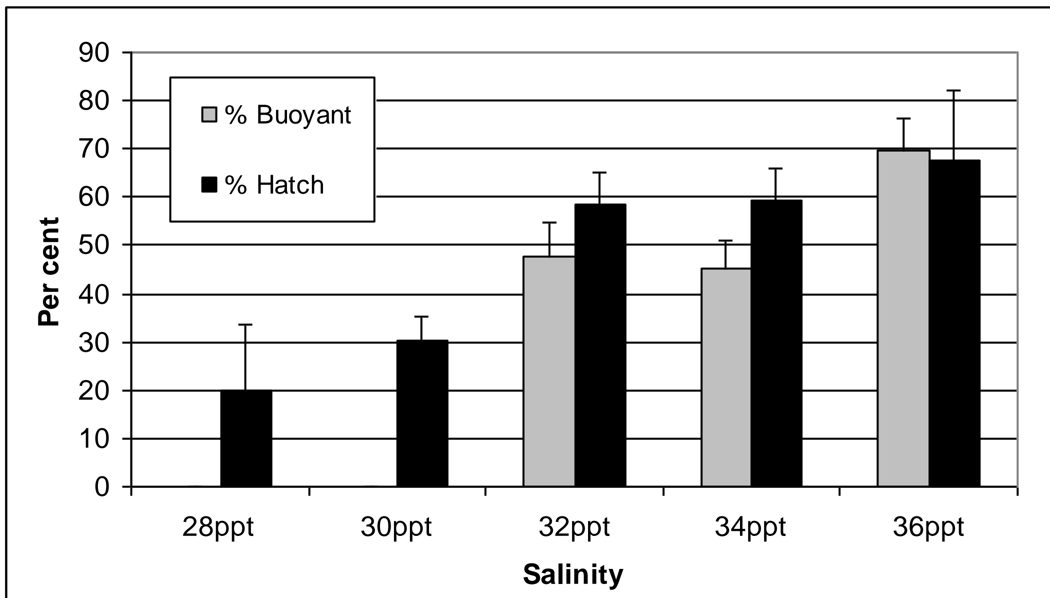


Figure 7: Effect of salinity on egg buoyancy and larval hatch rates. Error bars show one standard error of the mean (n=3) Note buoyancy calculations are only for fully buoyant eggs, not neutrally buoyant eggs.

Managing the incubation tank

At BIRC we run egg incubation tanks at full seawater salinities. Salinities lower than 32 ppt are avoided. If necessary, salinities will be increased to at least 32 ppt by addition of purchased sea salt (e.g. if local seawater supplies have been impacted by riverine flooding). Hatch rates are significantly lower below 32 ppt (Figure 6) and fertilised eggs lose buoyancy, making them difficult to separate from dead and unfertilised eggs and putting them at risk of bacterial contamination if they sink to the bottom of the incubation tank.

Tanks used for incubating eggs at BIRC are 1000 L volume, with a 1.1 m diameter. The bases of the tanks have a 7° cone. Incubation tanks are filled with 1 µm filtered, UV treated seawater. The tanks are heated to 28°C and **gently** aerated from the base of the central standpipe. Vigorous aeration can cause physical damage to jungle perch eggs. Up to 600,000 eggs can be kept in a 1000 L incubation tank. To maintain water quality in incubation tanks it is preferable to trickle filtered, UV treated water through the tanks. To prevent loss of eggs the central standpipe is modified to a cone shape (Figure 7) and fitted with a 250um mesh through which surplus water drains. Aeration at the base of the standpipe prevents eggs from adhering to the mesh. Tank water height is set by an external stand pipe system.

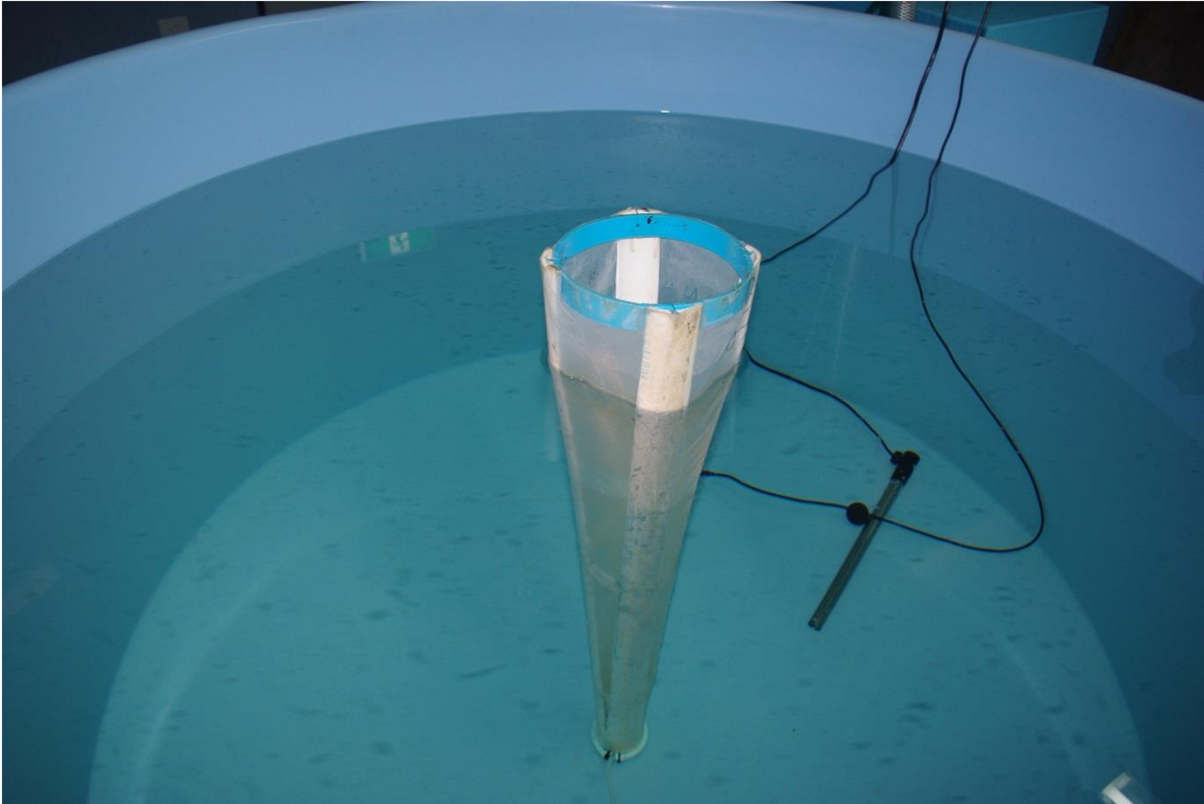


Figure 8: Egg incubation tank. Note the mesh covered conical central standpipe. In this photograph the aeration has been switched off leading to fertilised eggs rafting at the surface. Note aeration ring at the base of the standpipe.

At 28°C jungle perch eggs hatch approximately 15 hours and 15 minutes post fertilisation. To maintain water quality in the incubation tank it is best to remove any eggs that have died part way through the developmental process. To do this temporarily shut off the flow of seawater and the air supply, and switch off and remove any heaters. The water can then be gently swirled around the tank by hand and left to sit for approximately 10 minutes. With the aeration off and the swirling motion, any dead eggs will accumulate at the base of the cone, whereas most live eggs will raft at the surface (Figure 7). The next step is to siphon dead eggs from the base of the tank, then restart the air supply, water supply and, replace heaters in the tank and turn the tank heating back on.

Early stage jungle perch larvae are quite sensitive to handling and prone to physical damage. They are generally less than 2 mm in length at hatch. In contrast embryo stage eggs are much more robust to handling. Consequently BIRC staff have tended to move late stage embryos from incubation tanks to larval rearing tanks and ponds in preference to moving hatched larvae.

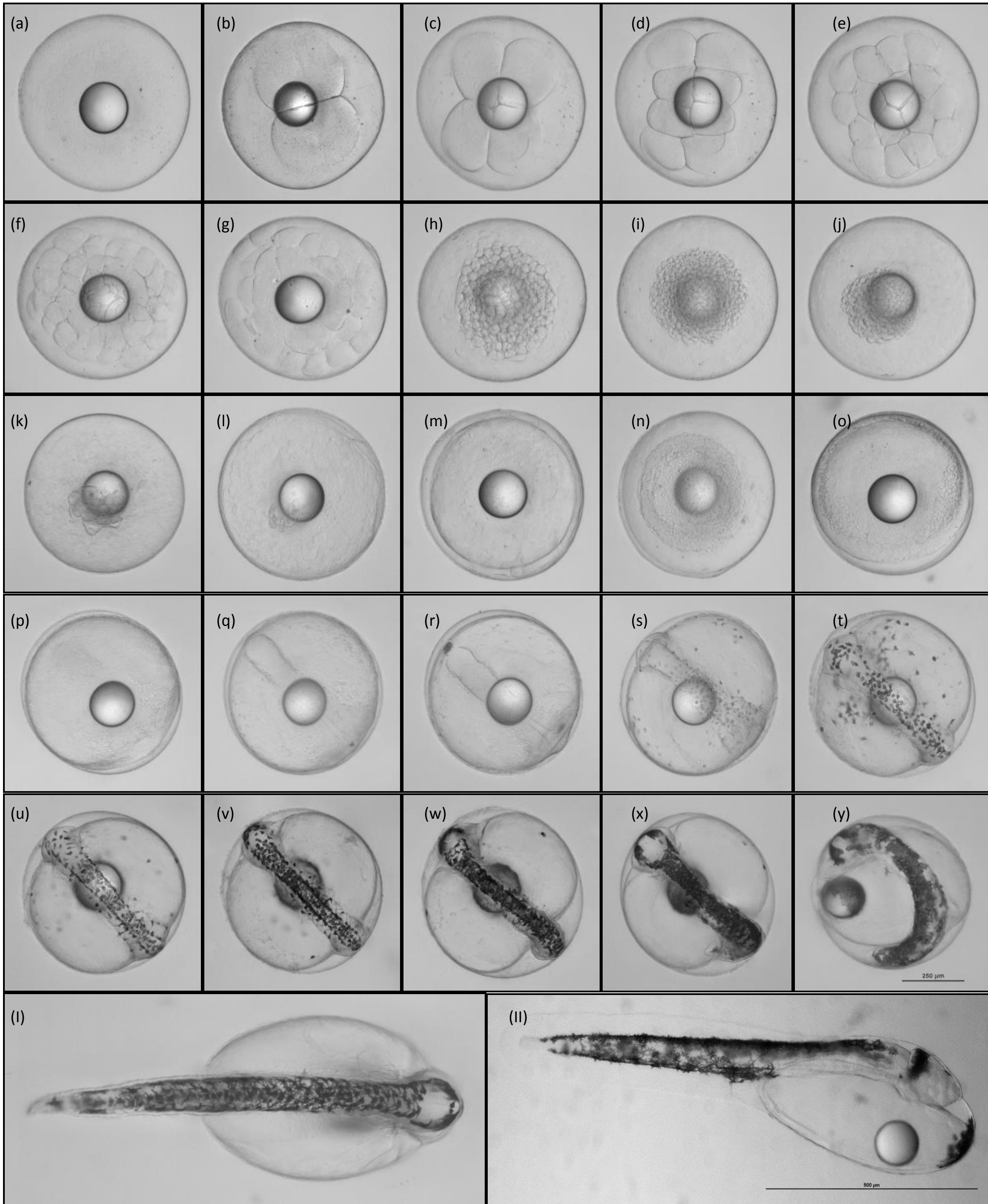


Figure 9: The stages of embryonic development for *Kuhlia rupestris*: single cell to late stage embryo (a-y) and I & II newly hatched larvae.

Collecting embryos and yolk sac larvae for distribution to larval rearing tanks

Embryo stage eggs are quite easy to remove from an incubation tank. Larvae hatch more than 15 hours after fertilisation. Embryos (stages u to y in Figure 8) are removed from the hatch tank around 10 to 14 hours after fertilisation. Embryo stage eggs are quite easy to harvest. Shutting off the air and water supply to an incubation tank causes live eggs to raft on the surface (Figure 7). Around 15 minutes after shutting off the air supply the majority of eggs will be rafting. These can be collected by skimming the surface water with a beaker or similar container. The methodology is best explained by viewing the attached video on egg incubation. Collected embryos can be placed in a bucket or tub of seawater and gently mixed. The number of eggs per mL can be estimated by pipetting several 1 mL samples from the mixed container and counting the embryos under a dissecting microscope as outlined for estimating egg abundance in the previous section. The total number of embryos in the container can then be estimated volumetrically. At BIRC we stocked embryos into larval rearing tanks at a density of 20 embryos L^{-1}. The appropriate volume of eggs to take from the mixed container to stock a hatch tank can be easily calculated. For example to stock a 1000 L rearing tank requires 20,000 embryos. If the mixed density of embryos in the bucket is 10 mL^{-1}, then 2000 mL of embryos would provide the desired number.

We generally stock ponds at much lower densities, because the natural production of plankton in the pond needs to keep pace with consumption of the plankton. The usual stocking density is 1 embryo or larva per 2.5-3 L (0.3-0.4 embryos L^{-1}).

It is not possible to remove all embryos from an incubation tank by the surface skimming method. Some embryos will be missed or displaced into the water column. There will always be some embryos that hatch in the incubation tank. For the first few hours after hatch, yolk sac larvae will raft at the surface. These can be collected using the same surface skimming method as for embryos. Be as gentle as possible because yolk sac larvae can be subject to physical damage. When larvae are no longer rafting (or to collect larvae that have been displaced into the water column), the water level in the tank should be drained down slowly through the central standpipe screen. The screen can be washed down gently to reduce the number of larvae adhering to it. Keep draining the water until it is level with the top of the base cone of the tank. Maintain gentle aeration in the water at this time. When the larvae are concentrated in the bottom cone, they can be gently scooped from the water using beakers or small plastic containers and tipped gently into a bucket or tub. Make sure some filtered sea water is in the bottom of the tub or container, before adding the first batch of larvae. It reduces the risk of physical damage if larvae are gently transferred into tubs by partial submersion. Keep scooping from the bottom of the tank until all larvae are collected. The number of larvae collected can be estimated volumetrically using the same methods as for eggs and embryos. Collected larvae can be transferred to larval rearing tanks or ponds, but make sure the combined density of larvae and embryos stocked in a tank doesn't exceed 20 L^{-1} and that the combined larval and embryo density stocked in a pond doesn't exceed 0.4 L^{-1}.

Link to egg incubation and management video

Live food production

It is feasible to do all larval rearing in ponds. If a hatchery opts to culture all stages of jungle perch larvae in ponds, then live feed production is optional provided copepod blooms can be sustained in ponds. However, even when not tank rearing larvae, live production of copepods can be useful to seed ponds and supplement natural copepod blooms in ponds.

Algae

To produce copepods it is essential to supply them with the micro-algae *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 large quantities (up to 300 L) of T-Iso in bags under controlled temperature and light conditions indoors.

Stock cultures

At BIRC stock cultures of all microalgal species were maintained axenically (i.e. without bacterial contamination) in 250 mL Erlenmeyer (conical) flasks in F medium (see Table 2 below). Stock cultures were gently swirled, at least once daily and sub-cultured using axenic techniques monthly or bi-monthly.

Microalgae culture (stock and working culture up to 10 L) at BIRC 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% shadecloth. 200L 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 2L 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 400mL, 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 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 (1000x) and added to cultures at a rate of 1 mL/L.

Table 2: Chemicals used for F media (A Trace elements, B Vitamins and C other chemicals)

A: Trace metals

mineral	chemical	formula	stock solutions
Cu	Copper sulphate	$CuSO_4.5H_2O$	10g/L
Zn	Zinc sulphate	$ZnSO_4.7H_2O$	22g/L
Co	Cobaltous chloride	$CoCl_2.6H_2O$	10g/L
Mn	Manganese chloride	$MnCL_2.4H_2O$	180g/L
Mo	Sodium molybdate	$Na_2MoO_4.2H_2O$	6g/L

B: Vitamins:

Name	Chemical	stock solution
Vitamin B12	Cyanocobalamin	1g/L (0.2g/200ml)
Vitamin H	Biotin	0.1g/L (50mg/500ml)
Vitamin B1	Thiamine hydrochloride	n/a

C: Other chemicals:

Names	Chemical	Formula
Nitrate	Sodium nitrate	$NaNO_3$
Phosphate	Sodium dihydrogen orthophosphate	$NaH_2PO_4.2H_2O$
Silicon	Sodium metasilicate	$Na_2SiO_3.9H_2O$
Iron*	Ferric citrate	$FeC_6H_5O_7.3H_2O$
	Citric acid	$C_6H_8O_7.H_2O$

*Iron stock solution must be autoclaved prior to use. See details below

Iron stock solution preparation (1 L):

- add 45g of ferric citrate and 45g of citric acid
- add distilled water to the 1000ml mark
- dissolve by autoclaving.

F Media concentrate (1000x) preparation protocols (5 L):

- add about 2 L of distilled water to a >5 L container
- add 750 g of Sodium nitrate ($NaNO_3$)
- add 10 ml of each of the 5 trace metal stock solutions
- add 10 ml of Vitamin B_{12} stock solution
- add 100 ml of Vitamin H stock solution
- add 1 g Vitamin B_1 (Thiamine hydrochloride)
- add 1 L previously autoclaved iron solution
- add 50 g sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$)
- top up to 5 L with distilled water
- when all chemicals have dissolved and the solution is well mixed, pour the media into five 1 L Schott bottles and store in the fridge.

Link to live food production video 1: T-Iso production

Copepods and copepod nauplii

Copepods are planktonic crustaceans. Copepod nauplii (the larval stage of copepods) were found to be the only suitable first feed for jungle perch larvae. Jungle perch larvae **will not take rotifers**, small strain (ss) rotifers or oyster trochophores. Jungle perch could not be successfully weaned from copepods to rotifers at any stage in trials at BIRC.

The production of copepods is based in large part on the methods developed for copepod production at the former Northern Fisheries Centre in Cairns (Department of Primary Industries and Fisheries 2011). The species of copepod reared as live feed for early stage jungle perch larvae in tanks is *Parvocalanus crassirostris.* There are some advantages to *P. crassirostris*. They 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 and the nauplii stages range from 40-100 µm (Figure 9). This means the nauplii are an ideal size for small fish larvae like those of jungle perch.

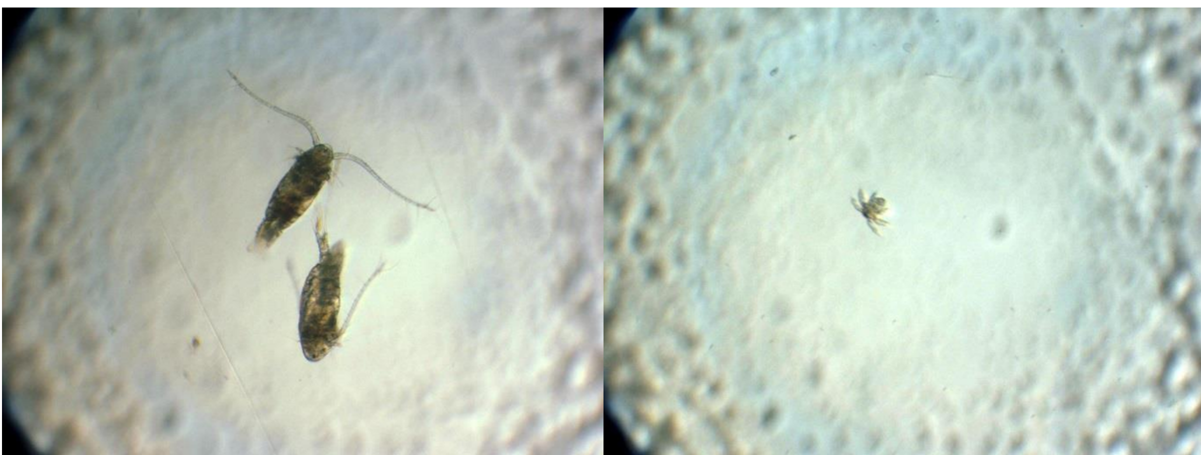


Figure 10: Copepod *Parvocalanus crassirostris* adults (left) and nauplius (right)

Avoid rotifer contamination

It is extremely important to keep copepod cultures isolated from any rotifer cultures. It is best if they are cultured in separate rooms. Equipment should not be shared between copepod and rotifer cultures. If working with rotifers, wash hands thoroughly before touching any equipment in the copepod room. If possible it is always best to work with copepods first, before working with rotifers on any given day. Working with rotifers prior to working with copepods increases the contamination risk. If rotifers get into a copepod culture they can quickly dominate it. To further minimise risk of contamination, copepod tanks should be kept indoors. This reduces the risk of wind borne contaminating biota.

Copepod tank setup and maintenance

Copepod cultures at BIRC are maintained in 1.1 m diameter 1000 L tanks with a 7° conical base. To prepare for a culture the tank is filled to a level of 300 L with 1 µm filtered UV treated seawater. Seawater treated by chlorination followed by dechlorination with sodium thiophosphate is to be avoided because **copepods are particularly sensitive to even small amounts of chlorine**. Therefore copepod culture is dependent on filtering and UV treatment to remove competing organisms and pathogens. The tank water is heated to 28°C and gently aerated. Lower temperatures can be used, but 28°C is considered the optimal rearing temperature for this species . Hang a scourer into the tank near the centre standpipe (Figure 10). The scourer captures flocculated algae and therefore helps to maintain water quality and also helps make the harvest process of copepods easier (see below).

Adult copepods are stocked into the tank at rate of 1 adult ml^{-1} using copepods harvested from another culture tank (Day 0). At the time of writing *P. crassirostris* start-up stock can be purchased from James Cook University, Aquaculture Department in Townsville. When first starting a culture from purchased stock the initial stocking densities will most likely be less than 1 ml^{-1}, but they will build up to the required density over one or two cycles. It is also possible to capture stock from the wild, but this will require careful picking of *P. crassirostris* from other copepod and plankton species to begin a pure culture. A number of on-line references describe how to do this, but it is far more convenient to purchase already cultured stock from established aquaculture research facilities.

After stocking the tank with adult copepods, add 15 to 20 L of the micro-algae (T-Iso) to the tank each morning. (Note the micro-algae *Nannochloropsis oculata* that is widely used for rotifer production is not suitable for rearing of copepods). The exact volume of T-Iso required will vary with the concentration of the *Isochrysis* culture. Aim for an algal cell density in the copepod tank of 5×10^4 cells ml^{-1} i.e. 50,000 cells ml^{-1}. At this density the copepod culture tank water should be visibly stained light yellow brown. If the culture is not very dense the volume added can be increased. Maintaining a suitable density of feed (*Isochrysis*) in the copepod tank is critical for copepod reproduction and nutrition. The scourer should be removed and washed in freshwater each day to remove accumulated flocculated algae, then placed back in the tank.

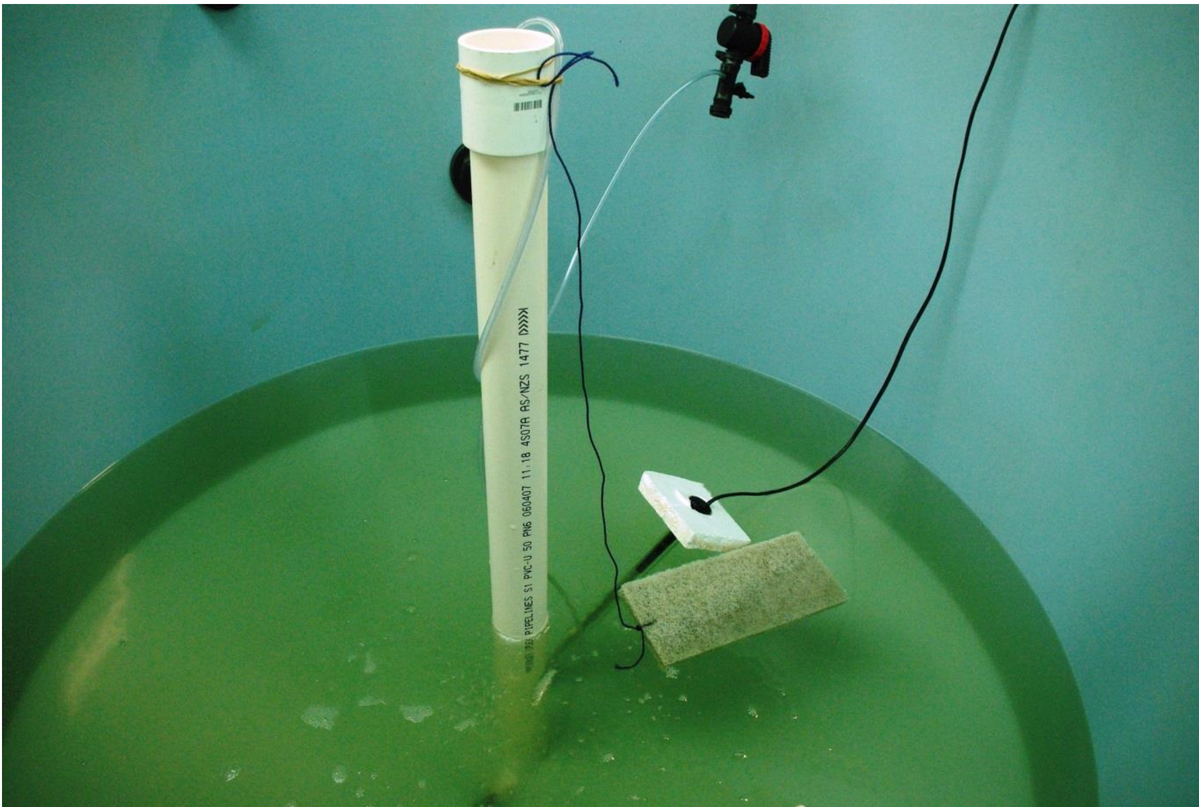


Figure 11: Copepod tank. Note the, gentle aeration, heater, water coloured by *Isochrysis* and the suspended scourer.

Monitoring copepods

If provided with adequate nutrition and heating, adult copepods should breed shortly after stocking into a tank. Nauplii should appear within one to two days after stocking (Day 1 or 2). Nauplii take around 7 to 9 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 should have increased to around 500 litres with daily addition of T-Iso, and copepod densities should have risen to between 3 and 10 ml^{-1}. Water temperatures should be monitored daily to confirm conditions are optimal. If the water temperature is too high or low, adjustments can be made to the heating regime. Air-stones should be checked to ensure they are still maintaining gentle aeration and mixing. Tanks can be run at different temperatures (e.g. 25 and 28°C) to enable provision of copepods for feeding at slightly staggered intervals.

Densities of copepod nauplii, copepodites and adult copepods can be easily monitored. This can be done by collecting a 100 mL water sample near where the air supply is upwelling water in the tank. Using a 2 mL pipette gently blow air into the sample to make sure it is well mixed. Use the 2 mL pipette to take a sub-sample from the 100 mL sample. Keeping fingers on the ends of the pipette (to prevent water loss) view the pipette under a dissecting microscope. Count the number of adults and other stages present. Repeat the process with another four 2 mL sub-samples. Calculate the mean number of adults and other stages present per 2 mL sample. Divide the result by two to obtain the mean number of copepods (or other stages) per mL.

From around day 9 some of the 100 mL sample can be placed in a Petri dish for observation under a dissecting microscope. If the majority of copepods are adults of even size and the male copepods are swimming with erratic quick movements, then the copepods are ready for

harvesting and separating into further cultures or are ready for stocking into jungle perch larval tanks.

If rearing jungle perch larvae in tanks, it is important to time spawning events around availability of copepods. Ideally copepod adults should be stocked into jungle perch larval tanks one day after the jungle perch larvae hatch. Copepod culture development rates can be slowed to some extent by decreasing the water temperature.

Harvesting copepods

Copepods can be harvested by draining the copepod tank water through a screen. The screen is set into a 200 mm diameter sectioned piece of PVC pipe (see Figure 11). The screened pipe is placed into a 20 L bucket with large drain holes cut approximately halfway up the bucket (Figure 11). These drain holes set the water level in the bucket to ensure copepods don't strand on the screen and also prevent the screened pipe from overtopping. The copepod tank is drained via flexible outlet pipe attached to a PVC pipe end piece. The end piece is an inverted U shape and tapers to a 25 mm outlet. The end piece hooks over the screened pipe (see Figure 11 and live food production video).

Figure 12: 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.

The copepod tank should be drained slowly (over 15 to 20 minutes) to prevent overtopping of the screened pipe. If scourers have been used in the copepod tank they will have reduced the amount of large particles in suspension and this in turn helps prevent the screen from becoming blocked. Twisting the screened pipe back and forth every few minutes helps prevent the screen from clogging and helps to keep the copepods in the water column.

Two different sized screens can be used. A 124 µm screen will collect only adult copepods. Other stages will pass through the screen. This screen can be used routinely when trying to harvest even-aged cultures to seed and build up new tank cultures. A 60 µm screen will capture most stages. Generally 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 all 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 harvesting copepods it is important not to expose copepods to air, especially the non-adult stages. Air exposure can affect moulting and development. This is why it is important to use the bucket draining system. When the tank has completely drained into the bucket, it is advisable to gently flush the screened pipe with 1 µm filtered UV treated sea water for about five minutes. This will remove any ciliates (that may compete for algae). If using a 124 µm screen the flushing will also remove any ss rotifers should there be any contamination. If your sample is contaminated with normal sized rotifers they cannot be removed using a 124 µm screen.

After flushing the sample, copepods can be collected by submerging small containers into the screened pipe in the bucket. The collected copepods can be transferred to a new culture tank, a jungle perch larval rearing tank or into a container of seawater for transport and release into a larval rearing pond. When transferring copepods to a new tank, release the copepods by partially submerging the container before gently pouring out the contents to prevent any air exposure (see video on live food production). Estimates of copepod numbers should have been made before harvesting the tank. Based on the estimated numbers the harvest can be split between two or more culture tanks, provided the stocking densities are somewhere between 1 and 2 adults ml^{-1}. For jungle perch larval rearing tanks adult copepod stocking densities should be between 1 and 3 adults ml^{-1}.

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 is quite straightforward and the process is usually adequately described on the product labels of the various suppliers. At BIRC we prefer to use Artemia that are supplied as pre-treated cysts impregnated with magnetic material. Use of a magnetic separator enables harvest of completely cyst-free Artemia nauplii (see live food production video). For untreated Artemia cysts the product labels normally describe alternative separation methods. These may involve use of screens or the attraction of Artemia nauplii to light. Untreated cysts also require treatment with chlorine solution or acid solution to hydrate the cysts for de-capsulation to improve hatch rates. This is not necessary with the pre-treated magnetised cysts.

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

The bottom outlet of the tank is then connected to an outlet drain which is run through a cylinder containing magnets (SepArt harvester) which overflows into a 125 µm mesh bag tied on the end of the cylinder and set into a bucket of seawater (see live food production video). The valve on the outlet of the egg-cup tank is then opened to allow a flow of approximately 20 L per minute through the magnetised tube system and into the bag. Artemia nauplii collect in the bag and any cysts are removed by the magnet system. After the tank has completely drained, the bag can be flushed with a flow of 1µm filtered, UV treated seawater. The bag can then be 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 are flushed into the bucket.

One 425 g tin of Artemia cysts should be sufficient to stock two 15 m x 15 m x 1.8 m (depth) ponds for two days. Approximately 250,000 nauplii are produced per gram of cysts. Artemia feeding is generally only required for a week to ten days while jungle perch larvae are weaned onto powdered feeds, and is only required if pond copepod blooms are waning. This is more likely in small ponds than in large ponds (see managing ponds for rearing larvae and fry below).

Link to live food production video 2: Copepods and Artemia production

Rearing larvae in tanks

Tank preparation and set up

Larval rearing tanks should be filled at least 24 hours prior to stocking with larvae or embryos. Prior to filling, tanks can be sprayed down with chlorine solution then rinsed out thoroughly with freshwater and left to dry. It is important to remove all traces of chlorine because copepods are intolerant of chlorine. The de-chlorinating agent sodium thiosulphate can also affect copepod growth. Dry tanks can be filled with UV treated 1 μm filtered seawater. The salinity should be at least 32 ppt, and preferably over 34 ppt. If necessary the salinity can be increased to desired levels by addition of sea salt. At BIRC we use 1000 L tanks filled to 600 L or 7000 L tanks filled to 2500 L for rearing jungle perch larvae. All tanks have a 7° conical base. The volumes used for larval rearing at BIRC have been limited by copepod supply, but if unlimited copepods were available all tanks could have been run at full volume. The water level in the tanks is set by 15 mm holes drilled part way up a central standpipe set within a 40 μm mesh covered outer cylinder (Figure 12). The 40 μm mesh is to allow water exchange in the tanks, whilst retaining all copepod stages and maintaining copepod densities.

Tanks are heated to between 27°C and 28°C. This appears to be the optimal rearing temperature for jungle perch larvae and it is also the optimal rearing temperature for *P. crassirostris*. The tank should be **gently** aerated near the central standpipe. Vigorous aeration can cause physical damage and also appears to interfere with feeding efficiency. Fluorescent lighting with daylight colour is set up over the tanks to provide an illumination level of at least 2500 Lux (Figure 12). Lighting enhances feeding success. Larvae kept in the hatchery under ambient lighting (illuminated only through a shade cloth covered translucent roof) failed to feed efficiently and all died by six days after hatch. In contrast those kept under bright light had much better survival (Figure 13). Artificial lighting is connected to a timer switch to provide periods of dark and light (see photoperiod below).

A surface skimmer is also set in the larval tank (Figure 12) to remove protein and oil from the surface. In large tanks two or three skimmers can be used. The design of skimmer used at BIRC was developed by Paul Palmer and consists of a well with narrow opening cut into dense foam. Air is directed through a nozzle made from a modified pipette to push surface material into the well. A similar design could also be made with plastic fittings. The skimmer is not activated until at least one day after hatch to avoid interference with rafting yolk sac larvae. Further details on skimmer use will be provided under the section on swim bladder inflation.

Probiotics can be added to the rearing tank water. At BIRC we add a probiotic containing a mix of *Bacillus subtilis*, *Bacillus lichenformis* and *Bacillus pumilis*. The probiotic is first added 24 hours prior to stocking larvae or embryos in the tank at the manufacturer's recommended rate of 10 g per m^3. Thereafter it is applied daily at a rate of 5g per m^3. The probiotic reputedly inhibits Vibrio and enhances waste degradation. We have not yet tried to experimentally validate the benefits of adding the probiotic to jungle perch larval rearing tanks, but anecdotally the water quality appears to be better in tanks where we have used the probiotic, compared to those where it has not been used.

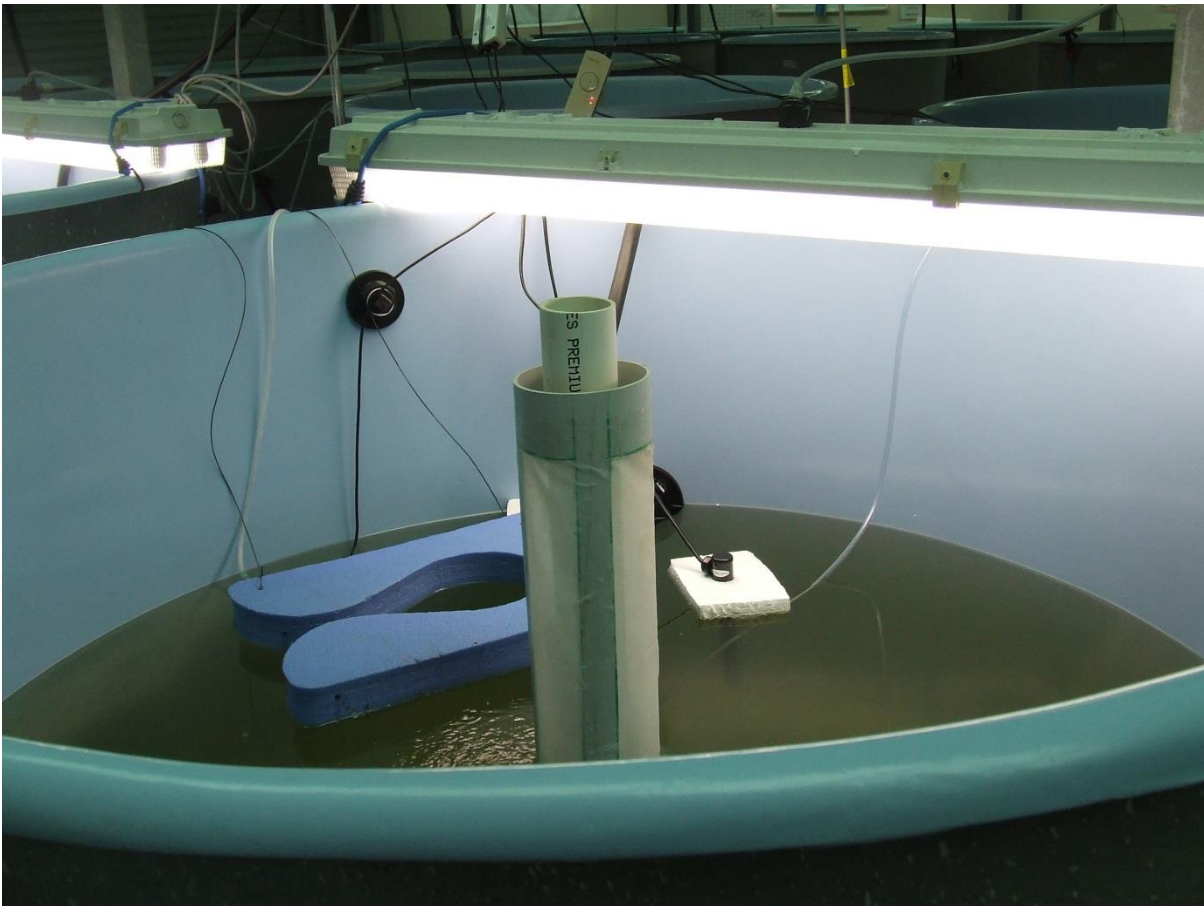


Figure 13: Larval rearing tank set up. Note central standpipe set in outer 40 µm mesh covered cylinder, overhead lighting, heater, gentle aeration and surface skimmer. The water is coloured by addition of T-Iso.

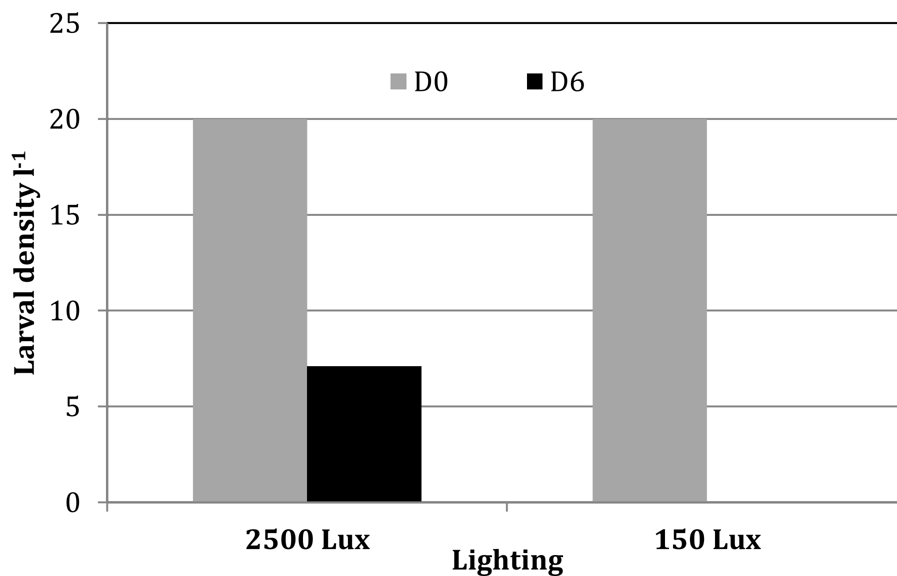


Figure 14: Effect of lighting on mean larval survival by 6 days after hatch (D6). Stocking densities on day of hatch (D0) were the same for each treatment. Number of replicates for each treatment =2.

Stocking tank

Yolk sac larvae or embryos can be stocked into the rearing tanks at a rate of 20 L^{-1}. Gently pour the embryos or yolk sac larvae into the tanks from a partially submerged bucket, beaker or similar container. The volume of embryos or larvae required to achieve the desired stocking density should have been calculated prior to release (see "Collecting embryos and yolk sac larvae for distribution to larval rearing tanks" above). The larvae will not be ready to feed for at least another 48 to 72 hours.

Siphoning to remove dead eggs and hatch waste

Around 24 hours after hatch and prior to stocking any copepods in the tanks, the base of the tank (cone) should be gently siphoned to remove any unhatched embryos, post-hatch egg fragments or dead larvae. Take care not to remove any live jungle perch larvae. At this stage the larvae should be evenly distributed in the water column but not very active. It is not possible to siphon once copepods have been introduced because copepod eggs are likely to sit near the bottom of the tank and any siphoning will interfere with nauplii production. The volume of water removed by siphoning should be minimal, but it can be replaced by slowly trickling in replacement UV treated 1 µm filtered seawater. Removal of unhatched embryos and egg shell waste is important to maintain water quality and to limit the chance of harmful bacteria growing on the base of the tank.

Photoperiod

Timers can be used on over-tank lighting to manipulate photoperiod. At BIRC we have had success with a 14 hour light, 10 hour dark photoperiod and we have also had good results from a 16 hour light 8 hour dark photoperiod. The longer light period provides extra feeding time.

Background Colours

Larval bowl experiments did not demonstrate any significant difference between general background colours (black, yellow, light blue and white) in terms of larval survival. We have had success using light blue coloured tanks. We prefer this colour because it makes it easy to observe the larvae and is not as stark or harsh as a white background, which can be stressful to fish. A trial was run to compare tanks with a granite (speckled) background with the standard blue background to reduce walling behaviour of fish larvae. Walling behaviour may have been reduced; however, the fish did not survive well with the granite background. It is not certain if this background reduced feeding efficiency or if the vinyl layer that was used to provide the granite background was slightly toxic to the fish. Similar vinyl layers to provide a granite background had been successfully used on yellowtail kingfish (Chen et al. 2014, Chen, personal communication).

Feeding larvae copepod nauplii

Green water culture techniques using the algae *Nannochloropsis oculata* and rotifers (e.g. Palmer et al. 2007) followed by weaning from rotifers to Artemia have been successfully used with a number of diadromous fish species, including barramundi *Lates calcarifer* and Australian bass *Macquaria* (*Percalates*) *novemaculeata.* Unfortunately this method was not successful for jungle perch larval rearing. Jungle perch larvae will not eat rotifers. If it is attempted to rear jungle perch larvae on rotifers they starve to death. All larvae are dead within four to five days post hatch at 28°C. It is *not* a case of them actually feeding on rotifers, and failing to receive adequate nutrition. Provision of enriched ss rotifers also resulted in larvae dying within four to five days post hatch. Survival of larvae was only achieved if they were provided with copepod nauplii as a first feed.

Adult copepods *P. crassirostris* should be introduced to the larval feeding tank 24 hours after jungle perch larvae have hatched. If the copepods have been harvested using 60 µm mesh, the sample may contain some non-adult stages, but this should prolong the availability of nauplii in the jungle perch larval tank. Stock the larval tank with a minimum density of one adult copepod mL^{-1} and preferably up to 3 mL^{-1}.

Just prior to or just after stocking adult copepods add T-Iso to the larval rearing tank. To maximise copepod reproduction and therefore nauplii output, it is best to provide a higher dose of T-Iso on the first day. To a 600 L tank add 40 L of T-Iso on the day of stocking. To a 2500 L tank add 160 L of T-Iso on the day of stocking. Thereafter 20L of T-Iso per day should be sufficient for a 600 L tank and 80 L per day should be sufficient for a 2500 L tank. The amount can be increased or decreased slightly depending on the density and quality of the T-Iso culture being used. A cell density of 5×10^4 ml^{-1} is desirable. However many hatchery facilities will not have the equipment for accurate algal cell counts. Suitable densities can be judged by eye. The source culture should be very dark brown almost black, and when added to the larval rearing tank the tank water should be visibly stained light yellow brown, but the bottom of the tank is still visible.

If colour is barely visible in the tank after the recommended volume of T-Iso is added and mixed in, your source stock may be too thin. This will make it necessary to add additional T-Iso to your feeding tank. There are a number of commercial algal pastes available that can also be used to feed copepods. These come with manufacturer's instructions. They may be convenient to use for small operations but can become expensive for very large cultures, compared to producing T-Iso on site.

Within a day of stocking adult copepods, copepod nauplii should begin to appear. Jungle perch larvae will be ready to feed late on the second day after hatch or around three days (72 hours) after hatch. The density of copepod nauplii can be monitored daily in the tank. Collect a 50 mL sample from near where the aeration is upwelling adjacent the standpipe. Use a pipette to collect 6 x 1mL samples and place these into sample wells or small petri dishes for viewing under a dissecting microscope.

Count the number of adults, copepodites and nauplii in each sample, then calculate a mean density per ml. Ideally the sample should exceed 1 nauplius mL^{-1}. Larvae have survived on just 0.2 nauplii per ml, but survival rates and growth rates are better with higher densities of nauplii. Most tanks should have nauplii densities in the range of 3 to 5 nauplii mL^{-1}. At times we have recorded densities as high as 10 mL^{-1}.

Larvae should be readily observed feeding on copepod nauplii. Mostly near the surface of the tank. The characteristic S shaped strike posture is good evidence that larvae are feeding. Overhead lighting seems to aggregate copepods, copepodites and some nauplii toward the surface and appears to aid feeding.

Swim bladder inflation

Swim bladder inflation in jungle perch larvae has been recorded as early as four days post hatch, but most inflation appears to occur between five and seven days post hatch (Figure 14). Anecdotally it appears that larger larvae are more successful at inflating their swim bladders. Perhaps these larvae are more able to push through the surface tension. Adequate densities of copepod nauplii should assist with larval growth rates and their ability to inflate their swim bladders. From 24 hours after hatch it is important to operate surface skimmers to collect protein and lipid waste off the surface. This waste will accumulate in the well of the skimmers and can be blotted off several times per day with paper towels, or cloth wipes. It is likely that the probiotic also helps with the breakdown of surface proteins and lipids. Keeping the water

surface clear of proteins and oils is important for swim bladder inflation. Larvae that fail to inflate their swim bladder are far less likely to survive. Those that do survive with uninflated swim bladders will have much slower growth rates as they are energetically compromised. They expend a lot of energy maintaining their position in the water column without an inflated bladder.

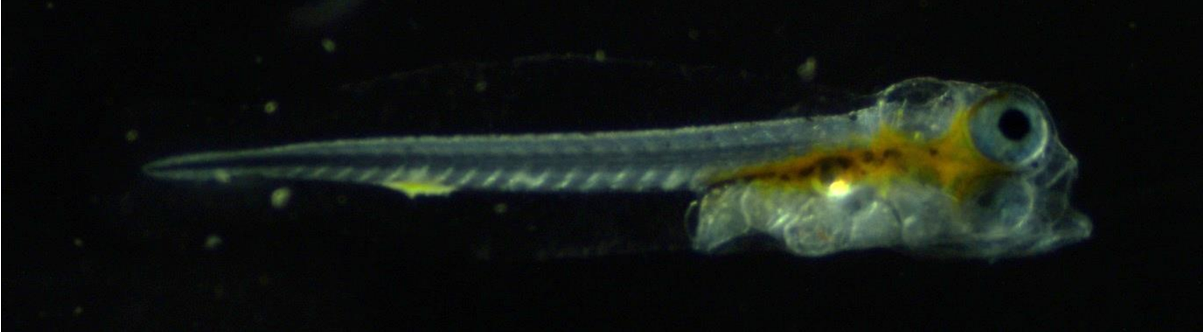


Figure 15: Jungle perch larva 7 days post hatch with inflated swim bladder

Water changes and screening

To maintain water quality, on day five after hatch it may be necessary to undertake a water change in the larval rearing tank. UV treated 1 micron filtered seawater can be trickled into the larval rearing tank. This will be permitted to drain through the central standpipe. As noted above the stand pipe should be fitted inside a 40 µm mesh screen. This will allow water to drain out, but will retain copepods, copepodites and nauplii. Replace approximately 50% of the tank volume with new water. T-Iso will pass though the screen, so it is essential to add fresh T-Iso back into the tank after the water change. If available (i.e. if copepod rearing tanks have been staggered at different temperatures) some additional copepods can also be stocked into the larval rearing tank at this stage in order to maintain reasonable feeding densities. The requirement for top up stocking of copepods will depend on prevailing densities of copepods, copepodites and nauplii in the tank.

Capture for transfer to ponds

If not transferred to ponds at the embryo or yolk sac stage, seven days after hatch larvae will be more robust for transfer to ponds. Seven day old larvae will be in the size range of 3-4 mm length. They will be able to consume larger prey items than first feeding larvae. Therefore nauplii or copepodites from a greater range of copepod species should be suitable for them to feed on. At this size, provided a pond has sufficient density of copepods there is an improved chance of survival in ponds. As seven day old larvae still tend to aggregate near the surface to feed, the majority can be quite easily captured by scooping from the surface with plastic jugs or beakers. These can be transferred gently to a bucket or tub. Make sure the bucket or tub is already partly filled with larval tank water before beginning this process. Remnant larvae can be captured by lowering the water level of the tank through the central standpipe screen to concentrate larvae into a smaller volume of water. Maintain gentle aeration during this process. The concentrated larvae can then be scooped out with plastic containers and transferred to the bucket or tub as above. Do not fill the bucket or tub to more than half full, before transferring it to a pond. The number of larvae in the bucket or tub can be estimated volumetrically.

Stocking ponds with embryos and larvae

Once the tub or bucket has been taken to the pond, add pond water gradually to the bucket or tub to allow the larvae to acclimatise to pond conditions. The bucket or tub should eventually be 50% pond water and 50% larval tank water. Float the bucket or tub in the pond for about 10 minutes for temperature adjustment, then gently submerge it into the pond to let larvae out.
It is important not to overstock a pond because too many larvae will quickly eat out their food supply. Larval mortality will be high in a pond, but it can be expected to achieve 2% to 3% survival of stocked embryos or larvae through to fingerling stage in a small pond and probably a slightly higher survival rate in a larger pond where plankton blooms are more stable.
Into a 15 x 15 m (400,000 L) pond stock 150,000 embryos or larvae. Into a 40 x 40 m (3,200,000 L) pond stock around 1,000,000 embryos or larvae, and into a 50 x 100m (10,000,000 L) pond stock up to 3,000,000 embryos or larvae.

Link to rearing jungle perch larvae in tanks video

Managing ponds for rearing larvae and fry

For most hatcheries access to saltwater ponds will be essential for successful mass production of jungle perch fingerlings. Jungle perch larvae do not wean from copepods to rotifers, so need to be maintained on copepods for at least three weeks. Production of T-Iso in sufficient quantities and captive rearing of copepods in sufficient numbers to sustain large numbers of jungle perch larvae prior to weaning onto *Artemia* would be too labour intensive for the majority of hatcheries. Ponds are a clear option for sustaining copepod blooms in sufficient quantities to rear jungle perch larvae through to fry and fingerling stages.

Pond preparation

Copepods are critical to successful production of jungle perch fingerlings. The objective of pond preparation is to promote and sustain a copepod bloom as long as possible. Ponds should be allowed to dry out before preparation. Three to four weeks prior to a proposed spawning, pond preparation can begin. Dolomite or lime should be spread over the dry pond bed. Application rates are shown in Table 3.

Table 3: 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 3,200,000 l	100 m x 50 m 10,000,000 l
Lime or dolomite application rate	5kg	40kg	120 kg

A mixture of inorganic and organic fertilisers should also be added to the pond. Initial fertiliser can be added to the dry pond bed or can be added as the pond is filling. The recommended mix of inorganic and organic fertilisers is shown in Table 4. Alternative organic mixes to those in the table can be applied, including animal manures.

The pond should be filled with raw seawater. Do not run the seawater through a sand filter because the objective is to introduce copepods and phytoplankton and a sand filter may remove them. A 300-400 µm screen can be fitted as a sock to the pond inlet pipe to remove fish eggs or fish larvae that may compete with jungle perch larvae, but this screen will still allow copepods, and phytoplankton to enter the pond.

When the pond is full, a paddle wheel or vigorous aeration can be used to help mix pond water and distribute fertiliser throughout the pond. However paddlewheels should not be used once embryos or jungle perch larvae have been added to the pond. After the initial fertilisation of the pond, follow up fertilising is required to sustain phytoplankton blooms and the copepods that feed on the phytoplankton. Recommended follow up fertilisation rates are also shown in Table 4. Follow up fertiliser should be applied at least twice weekly, until jungle perch larvae are weaned. Failure to follow up on fertilisation could lead to a premature crash of the phytoplankton bloom and corresponding crash in copepods.

Table 4: Pond fertilisation rates

	Pond 15 m x 15 m 400,000 l		Pond 40 m x 40 m 3,200,000 l		½ Ha pond 50 m x 100 m 10,000,000	
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 will generally enter the pond in raw seawater pumped in from the ocean or estuary. However the species composition and abundance of copepods pumped into the pond can vary according to prevailing conditions in the source water. To create more certainty in development of a copepod bloom, ponds can be seeded with cultivated copepods or copepods can be captured by plankton net from successfully blooming ponds and used to seed new ponds. At BIRC we have successfully seeded 400,000 L ponds with the copepod *Parvocalanus crassirostris*. Within a week of the initial fertilisation of the pond a phytoplankton bloom was apparent. At this stage two 600 L tanks of copepods were harvested (approximately 3 million copepods) 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 build up numbers naturally in the pond and supplement the natural multi-species copepod blooms from the raw seawater. *P. crassirostris* nauplii are an ideal first feed for jungle perch larvae and their presence in the pond helps ensure some larvae will have successful first feeds. Copepods will normally be taken to the pond in a bucket or tub of filtered seawater. It is best if this container is only half full, so that it can be mixed with pond water. This allows the copepods to acclimate to the pond conditions over a period of approximately ten minutes.

It is also possible to seed ponds with copepods from other ponds. For example a prawn pond containing prawns already weaned onto commercial pellet feeds may also sustain a bloom of copepods. Copepods can be captured from these ponds with a plankton net without impacting on the pond production. When harvesting copepods with a plankton net, transfer them to a bucket containing some pond water. Be careful to empty the plankton net into the bucket keeping the collecting end of the net submerged in the water. It is important not to expose copepods to air as this can prevent moulting and development of immature stages.

Monitoring the pond plankton bloom

Some ponds may take longer than others to build up adequate copepod densities and occasionally, ponds become dominated by rotifers and copepod densities do not reach sufficient densities to sustain jungle perch larvae. Jungle perch larvae have only survived in ponds at BIRC when the copepod nauplii density exceeded 120 nauplii L^{-1} at the time of first feed. As a general rule the higher the copepod nauplii density the better the larval survival. Densities of nauplii have approached 1000 L^{-1} at times in ponds at BIRC. It is quite common for rotifers to bloom in a pond initially, crash then be followed by a productive copepod bloom. Stepwise multiple regression of pond data at BIRC has shown that duration of copepod nauplii densities above 50 L^{-1} is the single variable that explains the greatest amount of variance in pond production of jungle perch.

There is no point stocking jungle perch embryos or larvae into a pond if there is an inadequate density of copepods and copepod nauplii. Normally copepod blooms have adequate densities of nauplii to stock jungle perch larvae within three to four weeks of filling a pond. However **it is best to postpone spawning until an adequate density of copepod nauplii is present.** This can be determined by monitoring the pond plankton bloom.

Monitoring the pond plankton bloom is relatively simple. In the weeks prior to stocking the pond with jungle perch larvae collect a 2 L water sample from the pond two to three times per week. If the pond is aerated collect the water sample near the aeration point as the water will be well mixed. Submerge a wide mouth 2 L plastic Nalgene bottle or glass jar into the pond around 50 cm below the surface. Take the sample to the hatchery laboratory for concentrating through a 20 µm filter (see the process in the pond management video). Concentrate the 2 L sample to 200 ml. Use a pipette to take six 1 mL sub-samples from the concentrated sample. Gently blow down the pipette to mix the concentrated sample before taking each subsample.

Place the subsamples into wells for viewing under a dissecting microscope. Count the number of copepod nauplii, copepodites and adult copepods. From the six sub-samples calculate the average number of nauplii per 1 mL sub-sample (count 1 + count 2 + count 3 + count 4 + count 5 + count 6) / 6. The same procedure can be used to calculate the average number of copepods and copepodites. Multiply the average count per sub-sample by 100 to estimate the total count per litre.

There may be several different species of copepods and copepod nauplii present. Suthers' and Rissik's (2009) book "Plankton, A Guide to Their Ecology and Monitoring for Water Quality" is a useful reference for identifying copepods and their nauplii to broad family or genera groupings. Nauplii less than 120 µm in size should be small enough to be consumed by first feeding jungle perch larvae. If the number of suitable sized nauplii exceeds 120 L^{-1}, then some jungle perch larvae are likely to survive. Ideally densities should exceed several hundred per litre, because the copepod bloom needs to sustain itself over approximately 2.5 to 3 weeks. As noted above stepwise multiple regression of pond data at BIRC has shown that duration of copepod nauplii densities above 50 L^{-1} is the single variable that explains the greatest amount of variance in pond production of jungle perch. Obviously duration of copepod nauplii blooms will also be linked to duration of blooms of adult copepods and copepodites on which more advanced larvae will feed. If copepod nauplii densities are suitable, and a series of samples has indicated a stable or upward copepod population trajectory then it should be safe to proceed with a jungle perch spawning.

After jungle perch larvae have been stocked into a pond it is important to continue to monitor plankton blooms in the pond at least twice per week. As larvae grow in size they will switch feeding from copepod nauplii to copepodites and to adult copepods. If copepod numbers show a downward trajectory and begin to approach a level of 50 L^{-1} or less then it may be necessary to supplement copepod numbers by stocking captive reared copepods or copepods from

another pond. If jungle perch larvae are large enough, then Artemia could be stocked as an alternative feed (see below). In larger ponds copepod blooms tend to be more sustained, so it is more likely that supplementation will be required in small ponds than in larger ponds.

Managing the plankton bloom and filamentous algae

To maintain the plankton bloom in the pond it is important to do follow up fertilising of the pond (see Table 4). The fertiliser helps to maintain phytoplankton blooms on which copepods feed. It is important to refrain from using lucerne chaff and pollard between days four and eight after hatch. During this time jungle perch larvae will be inflating swim bladders, and pollard and lucerne can sometimes promote scum formation on the surface that could inhibit swim bladder inflation.

Unfortunately regular application of fertiliser can also promote filamentous algae and *Ulva* (sea lettuce) growth. 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 begin to exceed 1 metre, then a non-toxic blue dye (Alpine Blue or equivalent product) can be added to the pond to help shade the pond bottom and prevent filamentous algae growth. The blue dye for ponds can be obtained from most aquaculture product suppliers. Apply the dye to the pond following the manufacturers' recommended rates. With aeration, the pond water will turn over and phytoplankton will still be exposed to the light in the surface layers and continue to bloom. Addition of blue dye can therefore help prolong phytoplankton blooms and copepod blooms. Some filamentous algae will probably continue to grow on the top 50 cm, of the pond walls, but this will not affect harvest of fingerlings, and can be easily physically removed by winding it around a stake or pole.

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 predate on copepods, and therefore impact on the larval food supply. Therefore as far as is possible, any hydras spotted in the pond should be removed. Those growing on the edge near the surface can be removed by hand. Larger hydras attached to the bottom can be scooped out with a coarse meshed long handled dip net.

Monitoring pond conditions

It is important to monitor pond conditions. At BIRC we monitor oxygen, pH, salinity, temperature and Secchi depth daily. Ideally pH should not exceed 9.5. If the pH rises above 9.5 it is possible to lower the pH by addition of molasses to the pond, but this has its own risks, because molasses can increase oxygen demand. If adding molasses additional aeration should be added to the pond.

If the salinity of the pond is less than 32 ppt prior to stocking with jungle perch larvae, then it may be necessary to add sea salt to the pond to bring the salinity level up. Survival is poor below 32 ppt for early stage larvae. If it doesn't rain much the salinity will gradually increase through evaporation. We generally prefer to have a starting salinity of at least 34 ppt, because it allows some margin of error if there is heavy rainfall shortly after stocking the pond. After a couple of weeks, larvae are generally more robust to drops in salinity. Jungle perch larvae have tolerated salinities of 38ppt, but if salinities start getting too close to 40 ppt (due to evaporation and lack of rainfall) we may top up the pond with fresh seawater or add some freshwater to bring the salinity back down to around 36 ppt.

If oxygen levels begin to fall below 4 ppt it may be worth adding additional aeration points or introducing some air lifts. Air lifts raise water through a 100 mm poly pipe with an elbow on top and create circulation of water around the pond and from bottom to top. We tend to leave air

lifts off in the first week of larval rearing, and just rely on air stones set on one side of the pond for maintaining oxygen levels. Early stage larvae seem to feed more efficiently in quiet zones, and do better if the air lifts are off. However, if oxygen levels are low in the first week, then it may be necessary to activate the air lifts. These can be set in all four corners of a pond. After day seven larvae are more robust to the gentle currents provided by the air lifts. Paddle wheels cannot be used at any stage as jungle perch larvae are surface oriented and likely to be injured by paddle wheels.

Monitoring the Secchi depth is also important. If the pond phytoplankton bloom begins to clear and Secchi depths begin to exceed 1 metre, then addition of dye can help restore the bloom and eliminate competition from filamentous algae (see above). In ponds that are not protected by bird netting, the dye can also help make fry and fingerlings less visible to avian predators such as herons and cormorants.

Monitoring larvae

If larvae are stocked into a pond as embryos or at the yolk sac larvae stage they will generally not be visible in the pond until at least day seven. Don't be disheartened if you can't see any larvae in a pond before day seven after hatch, this is the normal situation. Around day seven, yellow pigment begins to form over the gut region and this makes the translucent larvae more visible in the pond. The larvae tend to school near the surface. They will not generally be evenly distributed around the pond but aggregate in patches. To confirm there are larvae in the pond, walk the entire margin of the pond on a sunny day. They may be difficult to detect at first but once identified, they become easier to find. These aggregations are most likely to occur near where copepods or copepod nauplii are also aggregating. Larvae in ponds at BIRC seem to follow predictable daily movements around the pond, which seem to be related to the movement of the sun and prevailing winds. They tend to be on the eastern and northern sides of the pond in the morning, and the northern and western sides in the afternoons. Larvae are not readily visible on overcast days.

By closely observing the size of the larvae in the pond a grower can determine if they are at a stage where they may be able to take supplementary feeds such as Artemia or weaning diets (powders). Growth rates will vary according to pond temperature and prey density, but Artemia and weaning diets can generally be introduced around 20 days post-hatch.

Jungle perch larvae metamorphose into fry around one month after hatch. At this stage they can tend to sit a little deeper in the water column and be more difficult to see. Fry seem to favour sitting in and around aeration points. At this stage they may be feeding on invertebrates such as chironomid larvae carried on the aeration currents. Fry can also be supplementarily fed on weaning diets.

Supplementary feeding with Artemia and pelletised feeds

As noted above, if copepod numbers show a downward trajectory and begin to approach a level of 50 L^{-1} or less then it may be necessary to supplement copepod numbers. If jungle perch larvae are large enough, then Artemia can be stocked as an alternative feed. By 20 days post hatch most jungle perch larvae are able to take Artemia nauplii. One 425 g tin of Artemia cysts (250,000 per gram) should be sufficient to stock two 15 m x 15 m x 1.8 m (depth) ponds for two days. Artemia feeding is generally only required for a week to ten days while jungle perch larvae are weaned onto powdered feeds. Hatched nauplii can be released near aeration points in the pond or released adjacent to schools of jungle perch larvae (see pond rearing video). Stocking of Artemia is probably less necessary in larger ponds, but if the copepod bloom is waning, then supplementing copepods with stocked Artemia would be beneficial.

Concurrent with Artemia stocking (and even if not stocking Artemia) powdered weaning diets can be introduced into ponds. This is usually commenced around three weeks post hatch. Various weaning diets are suitable, including the O-Range and NRD diets. The initial weaning diets should be in the 0.2-0.4 mm size range. As the larvae grow and metamorphose into fry the larger weaning diets in the 0.4-0.8 mm size range can be gradually introduced. At BIRC we never totally eliminate the smaller size diet from pond feeding, but gradually mix in proportionally more of the larger diet size.

Feeding of weaning diets is done by loading auto-feeders in the morning and supplemented by a broadcast feed around the pond in the morning and again mid-afternoon. At BIRC in a 15 m x 15 m pond we use a single auto-feeder set adjacent to the aeration. In a 40 x 40 m pond we use 4 auto-feeders, one on each side of the pond. Each auto-feeder is loaded with 50 g of feed and a similar amount is broadcast to the total amount on the auto-feeders each feed. The amount fed can be gradually increased as larvae grow. For broadcast feeding we sometimes premix feed in a bucket of seawater to encourage sinking of the feed (see video). Post metamorphosis fry tend to sit deeper than larvae.

By four weeks post-hatch most jungle perch in the pond should be feeding on supplementary feeds. Supplementary feeding can continue until harvest. As supplementary feeding commences, the frequency of fertilising the pond can be reduced. The feed is adding nutrient to the pond. However it is still important to monitor Secchi depth, even though phytoplankton and copepod blooms become less vital as the fish grow. Preventing filamentous algae growth on the bottom of the pond remains a priority.

Harvesting fingerlings from ponds

Evaluating readiness for harvest

Dip-net some fry around the auto-feeder to determine their size. Fry are able to tolerate freshwater from around 18 mm in length, but they are not very robust to harvesting until 25 mm in length. If the majority of fry are 25-30 mm total length then they will be robust to handling and harvesting. This normally is between 50 and 60 days post-hatch. **Do not attempt to harvest fry if the majority are less than 25 mm total length.**

Drain harvest

At BIRC we drain harvest fingerlings from ponds. At more than 25 mm in length jungle perch fry are very robust to handling, and provided harvesting is done sensibly there should be few if any mortalities of fingerlings. Different ponds will have different drain harvesting systems. At BIRC larger ponds are drained through a screen set in front of drop (or monk) boards. The boards are removed from top to bottom until the pond is about 25 cm deep at the drop board side of the pond. At that stage the screen is removed and the remaining water drains through a large pipe to a trap set in a pit just below the level of the pond. Cement besser blocks set adjacent to drainage outlets are used to raise the water level in the trap to 30 or 40 cm depth.

In smaller ponds a 150 mm standpipe system is used. The usual standpipe is removed and replaced with a screened standpipe to allow the pond to slowly drain down. When the pond depth is about 25 cm deep near the drainage outlet the screened standpipe is removed and the water flows freely through a drain to a pit. A trap is set in the pit to capture fry that come through the drain. The trap is the same design as used in the large pond. As for large pond systems, cement besser blocks are used to dam the pit drain outlets and raise the water level in the trap.

The trap should be lined with soft mesh such as shade cloth to minimise damage to fry. The pond production video shows the trap system and use of besser blocks to raise water levels. Fry can be dip netted from the trap and transferred to buckets attached to ropes, which are hauled up and gently emptied into a fish transport tank system containing oxygenated seawater. At BIRC the constant flow through of water in the traps keeps them well oxygenated. Many private pond systems drain to a static sump set within the pond from which hatchery operators harvest fingerlings. **If using a static sump system it would be advisable to provide aeration to the sump during harvest**. Jungle perch are generally associated with flowing streams and clean water, so may have high oxygen demands.

Temporary holding of fish

At BIRC during harvest fry are held temporarily in a fish transporter. This consists of an insulated gel coated fibreglass tank set on the back of a trailer. The total volume of the fish transporter is 800 L. The transporter is connected to oxygen cylinders and oxygen is gently bubbled via regulators into the seawater in the tanks. A back-up pump aeration system is also connected to the tank. The tanks have lids to help prevent fry from jumping out and the lids also have screw top openings set into them, through which fry can be quickly transferred to the tank or monitored. When all fry from a pond have been captured or if densities in the fish transporters are getting too high, the fry are transferred to indoor 7,000 L or 10,000 L tanks filled with seawater.
Immediately after harvest fingerlings should be transferred to seawater tanks. The tanks should be well aerated and covered with shade cloth (30% shade) or 5 mm mesh to prevent fingerlings jumping out. The tanks should either be on flow through seawater or a recirculating seawater bio-filtration system. Fingerlings should be permitted to settle for 48 hours before transferring them across to freshwater. During this period they can be given light feeds of a commercial weaning diet in the 600-800 µm range or barra dust.

Link to pond rearing of jungle perch larvae and fry to harvest video

Rearing jungle perch fingerlings in tanks

Transition to freshwater

After the 48 hour settling in period, the seawater supply to the tanks can be shut off and freshwater allowed to flow into the tank at a rate of 5 to 10 L per minute. If using town water supply, it is best to run the water through an activated carbon filter to remove any chlorine. If using on site dam or tank water, UV treatment and filtration of the water is advisable to minimise risk of disease and parasite transfer to the fish. Eventually the tanks will become completely fresh. Alternatively the salinity can be allowed to drop to just 3-5 ppt and the tank systems then switched to a recirculating bio-filtration system.

Preventing jumping

Jungle perch are strongly attracted to inflowing freshwater. Fry just 25-30 mm in length can easily jump 50 cm high and through very small gaps. If inflowing freshwater is run in at the surface and creates splashing it will induce a jumping response at the inflow. To prevent loss and injury to fish it is best if the inflowing freshwater is directed to the bottom of the tank using a weighted hose or similar system (see linked video). This eliminates almost all jumping. It is also recommended to put a screen such as 5 mm mesh over the hose outlet, to prevent fingerlings from attempting to swim up the hose. It is also important to continue to cover the

tank with shade cloth or 5 mm mesh because some fish will still jump if startled or attracted to insects near the surface or other stimuli.

Feeding

Jungle perch fingerlings readily take commercial pellet feeds. Starting diets are usually in the 400-800 μm size range and can be gradually increased as the fingerlings grow. Any of the feeds normally used for rearing of barramundi fry and fingerlings will suffice for jungle perch fingerlings. At BIRC we broadcast feed pellets to jungle perch fingerlings two to three times per day. Fish are fed to satiation. Occasionally pellet diets can be supplemented with bloodworms or early stage instars of mealworms.

General tank management

It is important to maintain good water quality in the tanks. The fingerlings at BIRC are maintained on a recirculating aquaculture system, with UV treatment of the water, mechanical and bio-filtration. The tanks are well aerated. Alternatively tanks could be run as flow through systems. Given the small size of the fingerlings the tank outlets are screened to prevent escape. The 5 mm mesh screens must be cleaned regularly.

Frequent feeding of fingerlings leads to an accumulation of faecal matter and uneaten pellets on the bottom of the tank. This must be siphoned regularly (daily or every second day) to maintain clean conditions in the tank. Fingerling tanks at BIRC are heated. The temperature is generally maintained between 24 and 26°C, but may drop to around 22°C in mid-winter.

It may also be possible to rear jungle perch fingerlings in freshwater ponds on natural plankton and aquatic insect blooms, with some supplementary feeding if necessary. We have not tried this at BIRC. Pond reared fingerlings may be better suited to stocking as they will have more exposure to live feeds. The disadvantages of pond rearing may be poorer disease and parasite control and increased losses due to bird or dragonfly nymph predation. At BIRC losses of fingerlings in tanks have been virtually zero after transition to freshwater provided opportunities to jump have been eliminated.

At the time of writing this manual jungle perch are not a permitted species for stocking in Queensland waters. If they become a permitted species then there will be opportunities for hatcheries to supply fish stocking groups with fingerlings. In the meantime jungle perch could be supplied to the aquarium trade or trialled as an aquaculture species.

Link to rearing fingerlings in tanks video

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