GOVERNMENT OF WESTERN AUSTRALIA

TAFE WA North Regional

A summary of key hatchery processes for *Macrobrachium spinipies* (Cherabin)



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1.0 – General Information

In Australia a commonly used and accepted name for the freshwater prawn is cherabin. There appears to be two major species of freshwater prawn that are suitable for edible harvest and or edible aquaculture purposes, they are *Macrbrachium rosenbergii (*Australian strain*)* and *Macrobrachium spinipies*. It has been established that the species of cherabin that has been grown at Broome Aquaculture Centre (BAC) is likely to be *Macrobrachium spinipies* and not the previously suggested Australian strain of *Macrobrachium rosenbergii.*

It was initially proposed that the collection of wild gravid (berried) females from the Fitzroy river catchment (WA) would be the most reliable mechanism to facilitate hatchery-based production of cherabin post larvae (PL's) however, poor rainfall through wet seasons during the time of the project produced poor capture results and very minimal berried females were collected.

The Broome Aquaculture Centre developed systems they felt could allow the development of tank-based breeding to be tested and observed mating activity very quickly after implementing these systems. It was therefore proposed that where a lack of available berried females was evident, that captive breeding may be a viable mechanism to produce PL's for farming operations.

The following information relates directly to the culture of entirely hatchery reared cherabin juveniles, however it only represents work done at the Broome Aquaculture Centre 2017 – 2021 and should be treated as general information to help guide the decisions of businesses hoping to work with *M.spinipies* or other *Macrobrachium* sp. in Australia.

The systems described in this document were constructed of suitable and available equipment and, have not been wholly tested for their efficiency in producing sustainable volumes of juvenile cherabin.

2.0 – Broodstock management

Wild sourced broodstock were kept as breeding groups under ambient conditions in 3 raceways. The breeding groups comprised of 3 males and 9 females. Breeding occurred naturally around the full and new moons cycles typically during the wet season, November – March. Attempts were made to use photothermal manipulation to establish out of season spawning but no spawning activity was evident.

Animals were fed using a prawn broodstock diet manufactured by CP Feeds 2% biomass each day through the wet season and sparingly through the cooler dry season periods.

Supplemental feeding using bloodworms occurred three days per week at the rate of 1g per animal when water temperature was above 26^{o}C, below this the feeding of bloodworm ceased.

Post breeding the animals were monitored for embryonic development up to day 16 when eye-spotting of embryo's was evident and a change in egg colouration from a green colour to pale grey. Females were then added to a 20 x 10 x 10cm plastic mesh floating basket and moved to the larval rearing tanks.

Females were monitored for a period of approximately 2-3 days until hatching occurred. Some hatching occurred over consecutive nights, but most hatches were relatively synchronous. Females were placed back into the breeding tanks once hatching was completed.

A maximum of two females per hatching tank was used to stock the larval tanks to optimum stocking density of 60/L

Industry brood management practices are known to utilise extensively managed ponds systems to condition and breed broodstock. Emama Nguda trialled this technique and found that it may produce improve egg quality which could influence better larval survival. Their success showed that females recovered from the pond produced approximately 6,500 larvae/animal which reflects that of the best larval hatch rates from tank-based breeding. However, the comparative inputs to achieve the same relative performance could be lower than that to produce the same amount in tank-based system.

Male broodstock were identified to potentially reach the end of their life at approximately 2-3 years of age. This is unsubstantiated. However, a pattern of mortality was identified at BAC where dominant males died (for no apparent reason) approximately 2 years after being introduced to breeding systems.

A description of tasks is included in Appendix 1.0 – Broodstock Daily Procedure.

3.0 – Water quality management

Measurement and recording of water quality parameters should be undertaken on all systems at least once per day. Frequency of testing may need to be increased where poor water quality is identified, stress is observed, or mortality starts to occur.

Each system, broodstock, larval rearing and nursery, should be checked independently. Where recirculation systems are being used a sample may be taken from the sump tank. In systems that use batch culture techniques, each tank should be individually tested.

Table 3.1 indicates the expected optimum water quality ranges for Cherabin. These should be used as a strict guide to maintain a stress-free environment for the animals. Water exchanges on recirculating systems can be done in by topping up sump tanks with de-chlorinated freshwater or salinity adjusted saltwater, but normally occur to compensate for water loss through evaporation rather than strictly managing out of range water quality parameters. Water exchanges on the larval culture tanks will be needed more frequently to manage the water quality parameters within the optimum ranges.

Periodic assessment of Ammonia is recommended in larval rearing tanks particularly after stage three of development when the tanks begin to get dirty. Siphoning and disturbing the base of larvae tanks is not recommended as it may suspended particles that won't resettle. A twice daily drain of water from the bottom valve is recommended of up to 5L, this will clear some of the build-up and keep it in control. Make up water should be added at least once per day and should consist of water that is adjusted to target salinity and pH if they are out of range.

Table 3.1 – Optimum water quality parameters

Parameter	Acceptable Range
Temperature (ºC)	27ºC – 31ºC
pH	7.7 – 8.1
Dissolved Oxygen (mg/l)	5.8 – 6.8 mg/l
Free Ammonia NH^3 (ppm)	Not to exceed 0.02 ppm
Salinity	0ppt[B] / 6 - 10ppt[L]

[B] – Broodstock + PL's, [L] – Larvae

Aeration in the breeding systems should be applied evenly throughout the tank at a rate of 4 – 5L/min through an air diffuser. In larval tanks aeration should be supplied close to the based and middle of the tank using a diffuser at the rate of 2L/min and air should be supplied to nursery tanks at the rate of 4 – 5L/min but delivered around the based of the outlet screen using diffuse aeration line, this creates a slight air screen around the outlet preventing animals settling on the screen which can lead to blockages where higher numbers of animals are present.

Daily siphoning of PL (nursery) tanks should be undertaken twice daily where mortalities are present, this may be less frequent if mortalities aren't present. However, the siphoning to remove other wastes should happen every second day to prevent build-up from occurring.

All siphoning should occur into a harvesting bucket to prevent the escape of live or dead animals into waste-water outlets. Live animals maybe returned to the tanks if it is desired.

4.0 – Larval management

The larval phase of *Macrobrachium spinipies* is faster than that of other *Macrobrachium sp*. and metamorphose into post larval juveniles approximately ten days post hatch or after stage eleven. This means that technical staff need to be diligent in their observations and practices to ensure the cultures are well maintained and

Larvae were hatched and grown to stage ten of development in 250L artemia hatching cones. The hatching cones were used to facilitate good hydrodynamics of water in managing the suspension of live foods, the larvae, inert feeds, and algae pastes. Each cone was filled to 200L with ~8ppt saline water and appropriately managed through daily water quality monitoring. Target water quality parameters were managed to fall within the parameters shown in Table 3.1.

Trials have been undertaken to assess the performance of cherabin larvae in freshwater with limited success. Information suggests that some presence of saline water must exist to enable larval success however, it is unclear what the minimum, maximum and optimum parameters are for *M.spinipies* at this stage.

The larval culture process should use batched water processes to enable green-water culture's to have full effect. Salinity increases are likely to be the most variable parameter with the addition of

live feeds that are likely to be sourced from saline cultures. Risks associated with salinity can be reduced by adjusting salinity of incoming water changes to desired salinity and aging water prior to addition to culture tanks.

Additional information relating to the daily larvae work is shown in Appendix 2.0

4.1 – Larvae and mortality observations

Larvae numbers should be monitored daily by collection of samples from four random locations in each tank and then counted by eye over a dark background. Collection should be undertaken using a standardised technique. BAC undertook daily sampling by plunging an inverted 50ml or 100ml graduated beaker and righting prior to removing the sample from the tank. The method isn't reliable and could be a major source of error in establishing effective counts on larval Cherabin.

Estimated numbers of animals in the beaker can then be used to determine mortality and or survival and aide's decision making about the ongoing management of each batch.

A sample of 5 – 10 animals collected in the beaker should be observed under a stereo microscope for.

- Larval stage development
- Overall length estimate
- Gut Score
- Lipid Score
- Colouration
- Signs of deformity
- Mobility (not microscopy)
- Orientation (upside down or upright)

Appendix 4.0 – Daily observation and feeding record identifies the information that should be incorporated into procedures and records to assist in facilitating best practice observations of larvae. All observations should be routinely recorded on a pre-established record sheet.

Mortality observations should be undertaken twice daily from the bottom of each hatching cone. Draining water into a 10L plastic container should enable sufficient mortalities to be collected. The mortalities can be counted, estimating by eye, or directly counting. These counts should be recorded on daily observation sheets to aide decision making processes.

Additional aged water can be added to the tank post drain if deemed necessary.

4.2 – Larval feed maintenance

Larvae should be monitored for feed uptake at least twice per day. Counts of live feeds (Artemia) should be undertaken prior to feeding as this establishes the required additional concentration to add to achieve scheduled feeding targets.

Four samples should be taken from random locations within each tank using a 10ml pipette.

The technician should only add the required amount to achieve that indicated on the schedule, not what the schedule indicates, as this would mean an overfeed would occur. Examples of feeding calculations where surplus artemia exist are shown in appendix 3.0.

If using algae paste's they should be used sparingly so as to avoid polluting the water. Appendix 4.0 describes the inclusion of 0.5g of paste twice daily and is enough to add slight colour to the water. Pastes should be added and mixed with culture water taken directly from each culture tank. This aide's suspension of paste, preventing settling and associated water quality concerns with the use of pastes. Pastes should be checked periodically for the presence of pathogens.

> An unvalidated theory is that diatoms should be included in the larval culture enrichment and green-water processes. This may be supplied to the animals using pastes or live algae, but it has been suggested as a critical improvement to the larval feeding strategy that wasn't tested in the project.

To achieve consistent results with artemia processes it is important that all technicians undertake artemia work in a similar manner and developing a site-specific procedure from existing literature will ensure consistent results are achieved. Using the best quality artemia you can afford will also improve the consistency and reliability of the artemia.

Artemia Nauplii should be supplied directly to the larvae tank once they have been sanitised using the practices within section 6.2. Technicians should ensure that all artemia are rinsed thoroughly with clean saltwater through a harvesting screen prior to feeding. Where feeding day-old artemia they will need to be enriched prior to sanitising and feeding. Enrichment using instant bottled algae's is easy and relatively trouble-free, a maximum of 1ml of these enrichments per litre of water is recommended unless manufacturer instructions give different direction.

Successful enrichment of day-old artemia can be done within thirty minutes but it is suggested that the process should be undertaken over an hour to maximise enrichment uptake. Once the animals have been enriched, they should be rinsed of enrichment, sanitised and rinsed again with clean saltwater prior to feeding out.

5.0 - Post Larvae management

Under ideal conditions the time frame for post larvae rearing is approximately fourteen days before cannibalistic activity begins to have a negative impact on the survival of any given batch. The short period of time helps to reduce the risks with batch failure, but it does mean that staff should be sufficiently skilled and trained in recognising factors that can contribute to poor feeding

performance and be mindful of communicating all concerns to senior staff where immediate solutions can't be achieved.

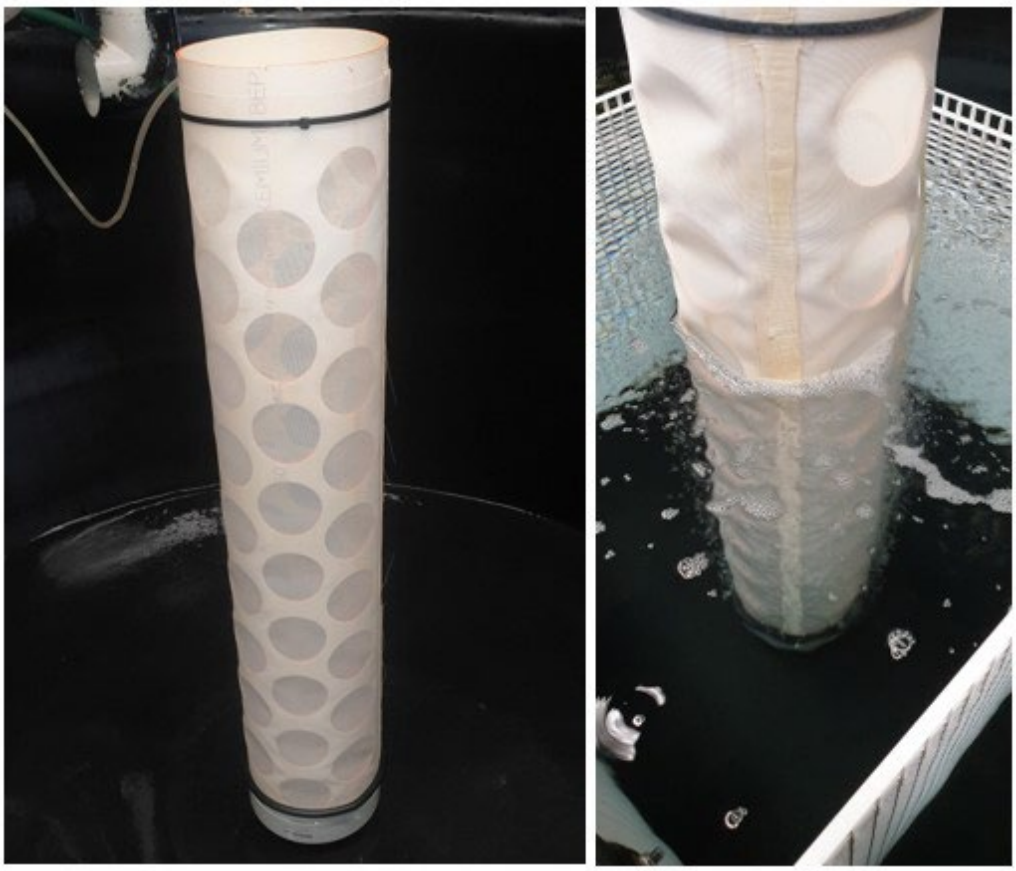
5.1 – Establishing nursery tanks

Tanks should be set up to allow for a gradual acclimation of salinity over about five days. Tank volumes at first stocking should be set up at 1/5 of the total volume to overflow, this allows for dechlorinated freshwater to be added at 1/5 of the volume each day for five consecutive days to bring the salinity down to approximately 0ppt. If the tank isn't at 0ppt by day five extensive addition of water can be made with strict monitoring of water quality parameters to prevent any acute changes.

A large bore central screen can be used on the main drain while a banjo screen can be used on the overflow if deemed a necessity. Screen sizes should approximately 180um or larger, this allows for small volumes of artemia to be flushed out of the system, enabling a stable and controlled supply of artemia in the tank, preventing excessive numbers of artemia from building up in the tank.

Air can be supplied to the tank via an aeration ring at the base of the outlet screen, this will create a mild air curtain which will assist in retaining smaller artemia and prevent cherabin from settling on the screen, preventing blocking.

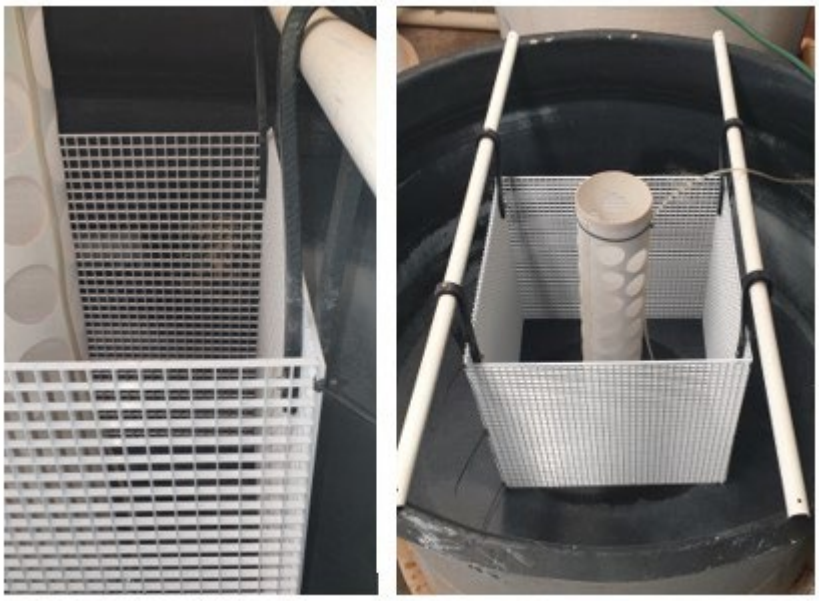
Image 5.1.1 – Nursery tank internal screens and air supply



To assist in moulting and minimising cannibalistic activity with the post larvae phase it is important to add a substrate to the tank which increases available surfaces for animals to occupy enabling greater stocking densities to be cultured. Ideally the substrate should be rigid and non-porous facilitating easy cleaning and deployment, they should be suspended to enable water to flow between the substrate and the tank base facilitating better cleaning when required. The substrate shown in image 5.1.2 is 20x20x18mm egg crate lighting panel used to diffuse artificial lighting in office spaces. Each panel is estimated to provide at minimum, an additional 39m^2 of available area for the juvenile prawns to occupy.

Ideally the first water added to the tank should be aged overnight which assists in stabilising water quality parameters prior to stocking larvae.

Image 5.1.2 – Suspended substrate



5.2 – Harvesting Larvae and Stocking the tanks

Larval development should be ascertained and if need a Larval stage index should be used to identify animals at stage ten of development prior to moving them. It's better to move animals prior to post larval metamorphosis as this has been demonstrated to reduce apparent cannibalism. Larval count procedures should be followed to establish approximate numbers that are being moved from the larval rearing environment to nursery environments. Densities of up to 10,000 larvae/m^3 (final volume) may be stocked without overly compromising survival and growth.

Test the water quality from the larvae and the nursery tanks and ascertain whether an acclimation is required or whether the water qualities align enough to enable the animals to be directly added to the nursery tanks.

To harvest the larvae the tank should be slowly drained over approximately an hour as this minimises disturbance to the tank and reduces the movement of waste particles to the harvester. When the tank is empty, gently rinse down the tank, trying to avoid disturbing wastes but rinsing off any larvae that might be stuck to the tank.

The harvesting bucket should use mesh screens of >200um - <300um which should enable solids to be removed but retain the larvae. Image 5.2 is an example of a harvesting bucket, note that the bottom of the harvesting bucket retains water so that larvae can be moved in water, reducing handling stress.

Image 5.2 – 1,000 µm harvester bucket



Once the harvest is complete gently rinse the harvester and larvae with clean dechlorinated freshwater to remove as much contamination and waste as possible. The animals can then be moved to the nursery. Animals should be gently released into the water by submerging the harvesting bucket as much as possible and gently rinsing down screens with water from inside the tank.

5.3 - Maintaining the water quality

Twice daily routine water quality checks should be undertaken each day and recorded on appropriate record sheets that incorporate the recommended range of parameters indicated in image 3.1. Daily ammonia monitoring is recommended but not essential until weaning and dry feeds are being used.

In the first five days post stocking the water quality is largely managed through the incremental additional of dechlorinated freshwater. After day five each tank should have reached overflow enabling the water quality to be managed using the recirculation system.

While the total biomass of each tank is rarely large enough to drastically impact the water quality it is still important that water quality parameters are assessed, and management decisions made appropriately. A decision-making process is included in Appendix 5.0.

A vacuum of tank bottoms, especially where waste build up is visible, should routinely occur morning and afternoon. Vacuuming should include the use of a screen to catch wastes (mortalities included), as well as any live PL's that are accidently sucked up. Vacuuming helps to keep abreast of feed wastes and metabolites ensuring water quality parameters are kept in range and reducing disease risks.

During the five-day acclimation process it is advisable to maintain a presence of algae in the water column. Maintaining a slight colour to the water is suggested as best practice with bottled instant algae's. It is suggested as little as 1g of instant algae/200L of water is sufficient. Determination of live algae concentrations should be undertaken at least daily where live algae is being used. Technical staff should seek to achieve an average cell density in the nursery tank of 250,000 cells/ml.

Figure 5.4 - Weaning schedule of Cherabin juveniles

Stage	Enriched Arts	Green water	Dry Diet 1	Dry Diet 2
10	10/ml	1g Iso/Shell	0.5g/day	0
11	10/ml	1g Iso/Shell	0.5g/day	0
PL1	5/ml	2g Iso/Shell	0.5g/day	0
PL2	5/ml	3g Iso/Shell	0.5g/day	0
PL3	1/ml	4g Iso/Shell	0.5g/day	0
PL4	1/ml	5g Iso/Shell	1g/day	0
PL5	0	Recirc	1g/day	0
PL6	0	Recirc	1g/day	1g/day
PL7	0	Recirc	1g/day	1g/day
PL8	0	Recirc	0	2g/day
PL9	0	Recirc	0	2g/day
PL10	0	Recirc	0	2g/day

Enriched day-old artemia should be maintained in the nursery tanks through the nursery period as indicated in the example (figure 5.4). The weaning process (PL3 – PL5) maybe shortened or extended if uptake of dry diets by the PL's indicates it may be needed. Commercially produced weaning diets are readily available for prawns, diet 1 (TNT200) and diet 2 (TNT300) were used by Broome Aquaculture Centre but not exclusively recommended for use.

Image 5.4.2 – Instant Algae used in larval rearing



Bottle 1



Bottle 2

Day-old artemia should be enriched with Iso 1800 instant algae (Bottle 1) or Shellfish diet 1800 (Bottle 2) or similar for a minimum of 30 minutes prior to being fed out. Enrichment should be added to the artemia at the rate of 1g/L of water and sufficiently aerated for the duration of the enrichment process. Enriched artemia should be rinsed using standard procedures prior to adding them to the nursery tank. Concentration of artemia in the culture system should be determined prior to feeding by taking four 10ml samples with a glass pipette and calculating an average of remaining artemia. Technicians should only add the difference between the scheduled feed amount and the existing amount. During weaning artemia feeds should be applied morning and afternoon while dry feed rations should be applied in even amounts across the day, like figure 5.4.1. Turning the air and water flows off during the application of dry feeds can assist in developing a strong feed response. Technicians should ensure all water and air supplies are turned back on prior to completing feeding activities.

Figure 5.4.1 - Daily weaning plan

Feed type	8:00	10:00	12:00	14:00	16:00
Live	100% Ration	-	-	-	100% Ration
Dry	-	33% Ration	33% Ration	33% Ration	-

Once weaning is complete dry feeds should be added to the tank three times each day, morning, early afternoon, and again late afternoon. PL's should be fed to satiety however, feed targets should be established based on 200µg/PL/day or 1g/5,000 PL/s/day as this will help control overfeeding and water quality problems.

Broome Aquaculture Centre had incorporated the use of Nanno 3600 instant algae with little success. It is known that Artemia selectively avoid Nannochloropsis algae as a food source therefore it isn't a suggested algae sp. to enrich artemia.

6.0 – Hygiene systems

It is paramount that hygiene is maintained through the use of clearly established procedures that are contextual to each hatchery environment and anything highlighted as important through environmental management documents. Training should be undertaken with all new staff to ensure they're aware of the possible factors affecting the biosecurity for each of the hatchery locations and its equipment.

6.1 -Tank management

Tank Cleaning principles align with that outlined in the AQUAVETPLAN operational procedures manual – Section 8.2 (Decontamination of fibreglass tanks). It is suggested that this process is followed using simple detergents and sodium hypochlorite solutions to minimise hazards associated with chemical use. The process of decontaminating larval culture equipment should occur immediately prior to stocking (1 – 2 hrs prior) and as soon as practical once larvae have been harvested to Post Larval systems. This allows for any immediate biosecurity concerns to be controlled once animals are move and any risks for accidental cross-contamination to be reduced prior to re-stocking with a new batch.

6.2 – Sanitation of live foods

While recommended, the sanitation of the live feeds could be avoided if strict hygiene practices are well established and adhered to throughout the production of the live foods and, it can be demonstrated that larvae cultures are free from aggressive bacterial outbreak from *Vibrio sp.*

During the artemia hydration process 1ml of sodium hypochlorite / L of water should be used to sanitise the outside of the cysts a thorough rinse is required to remove chlorine and all other waste products prior to stocking into hatching cones.

Post enrichment the Broome Aquaculture Centre used a weak formalin solution to sanitise the artemia but was only use out of convenience, other sanitation chemicals such as Hydrogen Peroxide may be of use. A formalin concentration of 50 ppm for a minimum of thirty minutes was effective at killing *Vibrio sp.* in batches of artemia prior to feeding out to larvae. A thorough rinse of sanitised artemia should be undertaken prior to feeding them out to remove all traces of formalin from the artemia.

6.3 – Sanitation of equipment

All equipment including, tanks, airlines and standpipes used in the artemia culture process should be sanitised post-harvest.

All equipment should be hosed or scrubbed to remove biofilms and other waste build up before adding to chlorine baths for a short period of time. Tank sanitation as mentioned in section 6.1. Chlorine baths should be made up to 1,000 ppm of active chlorine which enables equipment to be sanitised on contact.

Once sanitation is complete equipment can be hung up, stored appropriately, or allowed to stand to dry. If equipment or tanks need to be used immediately a thorough rinse to remove chlorine should be undertaken prior to use.

6.4 – Periodic bio-secure auditing

A regime to monitor and detect potential disease risks should be established and taught to all relevant staff members. *Vibrio* sp. exist as a potential contributor to mass early larval mortality and can be controlled using strict biosecurity procedures/hygienic processes. To test that these procedures are working water samples can be collected in sterile containers and then plated onto TCBS agar plates aseptically.

7.0 – Mortality and moult management

Cherabin can be quite aggressive, especially during the warmer breeding season, this does result in animal mortality associated with stress or direct predation (cannibalism). The highest mortality generally occurs during the moulting process or immediately after, when animals are still soft shelled. Providing adequate nutrition, monitoring animals stress, and maintaining sensible stocking densities will help control mortality in breeding tanks.

Under all circumstances dead animals and moults should be removed from the systems as soon as they're observed. This prevents the spread of any unidentified diseases. Moults and mortalities should be collected in a container the prevents leakage or spills.

At minimum a twice daily collection of larval and post larval mortalities from systems should occur. These can be taken by draining valves at the bottom of tanks or vacuuming mortalities from tank bases through a screened bucket.

Collected mortalities should then be handled and disposed of according to the business management plans. This may encompass the storage of small numbers by freezing in sealed bags and disposal through regular controlled waste collection.

Accurate counts and estimates of mortalities should be recorded each day, significant increases in mortality should be reported to management as soon as possible as management may need to escalate biosecurity contingencies or emergency plans.

8.0 – System description

Appendix 6.0 – Pilot scale cherabin hatchery depicts the layout of the systems used at Broome aquaculture centre throughout the project. The hatchery occupies a small area of approximately 61.50m^2 which suited the needs of the project. It is recommended for functionality and biosecurity reasons that the broodstock, larval rearing and post larval systems be established in their own zones.

The Broodstock system serviced the need to hold, condition and allow adults to mate through the breeding season. The main culture tanks are three fibreglass raceways each of

approximately 2.36m^3, two 1,500-watt submersible heaters were used to heat and stabilise temperature in the lead up to and out of the breeding season. Simple recirculation technology was utilised primarily to control water temperature at these times. A small, packed column biofilter (~45L) was installed to minimise risk associated with elevated levels of ammonia at higher water temperatures and feed rates. A 4,000L/hr submersible pond pump installed in a 200L sump provided sufficient water exchange and maintained water quality parameters within their required range. Water was supplied to each raceway through perforated 25mm PVC pipe this enabled the water to be sprayed into the tanks assisting in degassing and mechanical aeration. Top up water was added to the system as required using carbon filtered scheme water.

The larval rearing system was run successfully using batch culture techniques. Four artemia hatching cones were used as tanks, each tank had a total individual volume of ~250L however, the working volume for each batch was limited to ~200L. Air was supplied to each tank at the base of the cone via a 100mm carborundum air diffuser.

The post larval nursery system was established as a recirculation system primarily to conserve freshwater resources but also to assist in the control of temperature. The system comprised of a 400L sump, three 900L culture tanks each with a degassing tower water supply. An Onga 414 pump powered the recirculation through two filtration lines of bag filtration (~1,000µm) and a ~45L packed column biofilter. The pump over supplied water to the system, even at capacity so a useless loop to the sump was utilised to reduce line pressure. Water levels to the system were maintained using a float valve, periodic assessment of it's function was needed to prevent failure. The recirculation of tanks in the nursery only occurred after freshwater acclimation of juveniles was achieved.

9.0 – Recommendations

Reason	Recommendation
Poor quality hatching	Don't move female to larvae tanks until after eye-spotting can be confirmed, this reduces stress in handling baskets.
Improved egg quality	Try to include fresh supply of fatty and amino acids in wet diet e.g. bloodworms, mussels.
Improved egg quality / Improved larval survival	Incorporate collection of animals from broodstock ponds.
Improved larval survival	Ensure hygienic processes are established for larval culture including live food hygiene and system sanitation.
Improved larval survival	Incorporate Isochrysis and Diatoms into green-water culture and live feed enrichments, limit Nannochloropsis sp. especially with artemia enrichment.
Improved larval survival	Harvest and move stage ten larvae and stock into prepared juvenile rearing tanks.
Improved juvenile survival	Ensure sufficient substrates are used in PL tanks and time to harvest to farm occurs within 14 days post metamorphosis.
Improved juvenile survival	Ensure some artemia are present until direct observation of dry food consumption can be made.
Improved juvenile survival	Seek options to grade larvae away from PL's in nursery phase.
Broodstock management	Routine collection of breeding animals from the wild to account for breeder mortality
Broodstock management	Keep an accurate count on stocking density and breeder ratios
General batch performance	Allow staff sufficient time to undertake live works.

Appendices

Appendix 1.0 – Broodstock daily procedure

1. Review the stock in the system to see if there are any mortalities, new moults or if any females show signs of holding eggs. Record on the datasheet any observations. **(It is important to record the first day females are seen holding eggs so the date of hatch can be estimated).**
2. Quickly drain water from the valves at the end of each raceway to remove anoxic water.
3. Check that all WQ meters to be used are functioning and have been calibrated as required. **(The salinity on the D.O meter should be set to 0ppt).**
4. Using a jug, take a sample of water from the sump of the system.
5. Test the pH, D.O and salinity of the water in the jug, recording measurements on the datasheet.
6. Check the Thermometer hanging in R3 Raceway and record the temperature.
7. Top up the sump **daily** so it is full of a de-chlorinated, fresh water supply located near the Ornamental heater/chiller if required.
8. If there is considerable waste on the floor of the raceways, use the vacuum hose and remove it from the raceways. (**This should be done at a minimum of once a week).**
9. Review the feed schedule located on the wall of the workbench and prepare the required feed for each raceway individually. The food will be located in shed 2's fridge/freezer.
10. Evenly distribute the feed throughout each of the raceways.
11. Record the amount of feed added to each raceway on the datasheet.
12. Wash any equipment used with fresh water and place it back in its designated place to dry.
13. Hose down the floor with fresh water towards the drain to remove dirt and waste.
14. Report any concerns or observations to the Hatchery Manager.

Procedure

- **a)** Identify female brood stock cherabin with broods (clutches) of eggs and their stage of development.
- **b)** Prepare hatching tanks, first feeds and transfer brood stock.
- **c)** Carry out daily larval activities until PL as per schedule.
- **d)** Count and transfer PL's to nursery tanks.

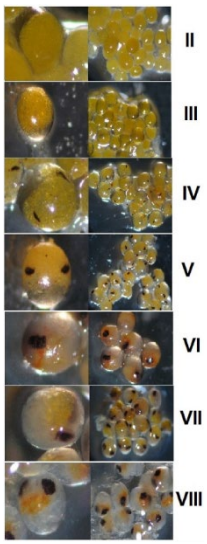
a) Identify gravid females and egg development stages:

Embryos of cherabin generally develop in eight sub-stages before hatching (see below).

While they are attached to the female, they will appear to go through four distinct colour changes:

1) White/yellow
2) Rich yellow
3) Yellow/green with eyespots forming
4) Green/grey with eyespots and white stripes (embryo tail section forming)

Image A2.0 – Colour and embryo development of *Macrobrachium Americanum*



Source : Sainz-Hernandez, 2016

b) Tank preparation:

1. When female clutches are rich yellow, prepare hatching tanks (250L conical) by chlorinating according to standard dosage for 24hr, then rinse with fresh water.
2. Let tank air dry for a further 24hr
3. Then fill to 200L with 8ppt brackish water and aerate. One tank per gravid female is required.
4. Females that are carrying eggs of green/grey eggs with black eyespots are ready to move to a hatching cone

5. To move the female gently capture her and avoid dislodging eggs as much as possible.
6. Place the female into an individual floating cage until the eggs hatch
   prepare required amount of Artemia for hatching to enable first feed of Cherabin larvae. This is done on the day of brood stock transfer.

c) Larval rearing activities:

Preparation of Artemia feeds –

1. Artemia are prepared a minimum 24hr in advance of the feeding schedule.
2. Ensure Artemia that need to be enriched are prepared 48hr in advance of the feeding schedule.
3. Follow SOP #2 Artemia culture manual for the relevant processes
4. Artemia for Cherabin feeds will need to be hatched in salt water (See part 3.1 of the Artemia culture manual)

Mornings –

5. Referring to the record sheet, conduct and record standard water quality measurements.
6. Perform a visual observation of the larval rearing tank
7. Then drain water from the bottom valve of each tank into a 1 L container.
8. Estimate the number of mortalities retrieved from each and record of the data sheet.
9. Take 4 x 500ml water samples from the top of each larval rearing tank and count the number of live larvae.
10. Calculate the average of the 4 x 500ml samples
11. Multiply the average by 500 to get the estimated number of larvae in the tank and record on data sheet.
12. Take 5 larvae from the larval rearing tank and observe under a dissecting microscope. Record average stage of development, TL and condition ratings. A chart is provided in Shed 3 to help identify larval substages.
13. Take 4 x 1ml water samples and find the average number of Artemia remaining in the tank. Top up to the required amount on the schedule with the hatched or enriched Artemia.
14. Add required algae paste to larval rearing tank according to schedule.
15. Change and clean screens according to schedule.

Afternoons -

1. Perform a visual observation of the larval rearing tank, then dump the bottom valve of the tank into a 1 L container. Estimate the number of mortalities retrieved and record of the data sheet.
2. Take 4 x 1ml water samples and find the average number of Artemia remaining in the tank. Top up to the required amount on the schedule with the hatched or enriched Artemia.
3. Add required algae paste to larval rearing tank according to schedule.

Appendix 3.0 – Live feed calculations

Where surplus artemia are identified in larval cultures it is necessary to adjust the amount of additional feeds to limit excessive feeds that place pressure on controlled supply of algae pastes. The following examples are calculations that can be used to determine the amount of live food (artemia, arts for short) to add to a larvae culture to achieve the recommended feed concentrations.

Scenario One

The required concentration of artemia is 10arts/ml, you have counted 2/ml. The culture tank is 200L or 200,000ml.

- 10 arts/ml – 2 arts/ml = 8 arts/ml
- 200,000ml (tank volume) x 8 arts/ml = 1,600,000 arts total

Scenario Two

The required concentration of artemia is 10arts/ml, you have counted 6/ml. The culture tank is 250L or 250,000ml.

- 10 arts/ml – 6 arts/ml = 4 arts/ml
- 250,000ml (tank volume) x 4 arts/ml = 1,000,000 arts total

Scenario Three

The required concentration of artemia is 10arts/ml, you have counted 1/ml. The culture tank is 150L or 150,000ml.

- 10 arts/ml – 1 arts/ml = 9 arts/ml
- 150,000 (tank volume) x 9 arts/ml = 1,350,000 arts total

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Appendix 4.0 - Daily observation and feeding record - Front

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2020 Cherabin Run Schedule based on 5000 Zoea in Static 200L Cone

Date:

Batch:

Length	Stage	Rotifers maintain @	Artemia Cysts (g) to Hatch	Artemia Stage	Artemia maintain @	TNT128 / g / day	Feeds / day	Algae Paste Nanochloropsis sp. / Shellfish mix	Salinity (ppt)	Water ex (%) / Day	Daily System Information.
	-1	-	8	-	-	-	-	0.5g/0.5g	8		*Tank filled to 150L @ 6ppt. Cap exit pipe, airstone to base.*
	0	-	8	Nauplii	5/ml	-	2	0.5g/0.5g	8		*Cap exit pipe, airstone to base.*
	1	-	8	Nauplii	5/ml	-	2	0.5g/0.5g	8		*Cap exit pipe, airstone to base. Top up to overflow height at 8ppt*
	2	-	26	Nauplii	5/ml	-	2	0.5g/0.5g	8		*Cap exit pipe, airstone to base. Split arts 50:50, half to enrich as day old, half to immediate feed*
	3	-	13	Nauplii	10/ml	-	2	0.5g/0.5g	8		*Cap exit pipe, airstone to base. Enrich day old Arts Iso*
	4	-	13	Nauplii	10/ml	-	2	0.5g/0.5g	8		*Cap exit pipe, airstone to base. Enrich day old Arts Iso*
	5	-	13	Nauplii	10/ml	-	2	0.5g/0.5g	8		*Cap exit pipe, airstone to base. Enrich day old Arts Iso*
	6	-	13	Enriched	10/ml	-	2	0.5g/0.5g	8		*Cap exit pipe, airstone to base. Enrich day old Arts Iso*
	7	-	13	Enriched	10/ml	-	2	0.5g/0.5g	8		*Cap exit pipe, airstone to base. Enrich day old Arts Iso*
	8	-	13	Enriched	10/ml	0.5	2	0.5g/0.5g	8		*Cap exit pipe, airstone to base. Enrich day old Arts Iso*
	9	-	13	Enriched	10/ml	1	2	0.5g/0.5g	8		*Cap exit pipe, airstone to base. Enrich day old Arts Iso*
	10	-	13	Enriched	10/ml	1	2	0.5g/0.5g	8		*Full harvest, count and move to 900 PL tanks*

Artemia

Disinfect cysts in 2ml Chlorine / L water for 30min and rinse well.
Hatch in 6ppt salinity @ 29ºC.
Extra Artemia can be stored aerated in fridge overnight for use the next day.
Artemia Nauplii to be sanitised in 50ppm formalin for min 30min
Sanitised Artemia Nauplii to be rinsed with clean saltwater prior to feeding

Water Quality

NH_3 - Not to exceed 0.019ppm, test as requi
pH - Maintain 7.7 to 8.1.
DO - > 5ppm
Temp - 27-31ºC.

Daily Tasks

Feed counts and additions before 9am and after 3pm.
Algae paste additions twice daily.
Counts on 4 known volume samples and average to determine stocking density.
Dump valve at base of cone to remove mortalities twice daily.
Perform larval operations on 5 individuals (length, condition, stage).

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Appendix 4.0 - Daily observation and feeding record - Back

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Cherabin Larval Observations								
	CH Cone No:		Batch:	Condition Observations				
Date	Daily Counts Per 50ml or 500ml sample.	Total Length (mm) TL 5 Observations and Mean	Life Stage 5 Observations and Mean	Gut Cont. 0, 1, 2	Lipid 0, 1, 2	Colour B, O, R	Mobility 0, 1, 2	Comments

Appendix 5.0 – Water quality management decision making process

WATER CHEMISTRY RELATIONSHIPS AND MANAGEMENT LIMITS FOR CHERABIN CULTURE					
	ACTION LEVELS			RECOMMENDED ACTION	
	Target	Caution	Severe		
Temp	28 - 31	<26, >32	<23 - >33	If temp low reduce feeding, remove shade. If high increase shade and keep pump running 24/7.	If very low stop feeding and add heater. If very high stop feeding, shade system and provide some cooling system.
DO	6	<5, >8	<4, >9	If low check aeration system, vacuum tank, clean sump and filter, reduce feeding. If high check for supper saturation.	If very low stop feeding, increase aeration, do a large water exchange, clean system. Check degassing is staking place.
NH_3-N	<0.02	0.02 - 0.03	>0.03	Vacuum tank, clean filter screens, allow pH to drop to caution limits.	Stop feeding until <0.025. Water exchange and clean system.
NO_2-N	<1	>3	>5	Add NaCl to achieve 10:1 ratio to nitrite, 1ppt to every 1ppm of nitrite.	Skip Feeding until <5; increase water exchange Maintain elevated NaCl.
NO_3-N	<60	100-300	>300	Increase water exchange until <100. Or with batch cultures calculate require volume to remove and dilute with dechlorinated freshwater	Increase daily water exchange or calculated batch dilution and stop feeding until <100.
pH	6.8-7.8	<6.7-7.9>	<6.1->8.5	If low, increase to top of alkalinity scale, increase aeration. If high, Reduce to bottom of alkalinity scale and reduce aeration.	Adjust with Acid or Base (max adjustment 1 unit per day)
Sal. (PPT)	0	2-5	>5	Top up sump with new water, test incoming water for salinity.	Perform large water exchange, calculated volume to bring salinity back to target

Appendix 6.0 - Pilot scale cherabin hatchery

