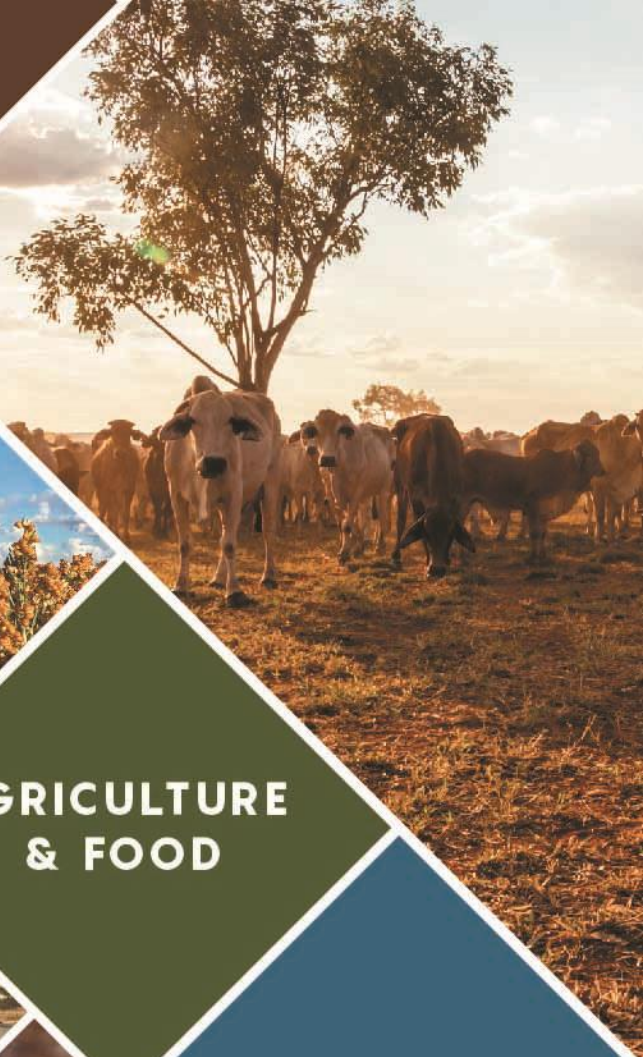
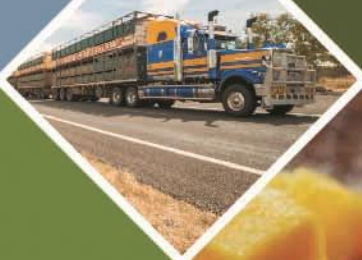
NORTHERN HEALTH SERVICE DELIVERY

TRADITIONAL OWNER-LED DEVELOPMENT

AGRICULTURE & FOOD





AT.2.1718098 Cherabin aquaculture production as an enabler for Indigenous business development

Final project report

North Regional TAFE

CRCNA

DEVELOPING NORTHERN AUSTRALIA

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Contents

1.0 Project Background

The project was initiated late 2017 where training was to be provided by North Regional TAFE's Broome Aquaculture Centre (BAC), to a cohort of students at the Mud Springs aquaculture site owned by Ribinyung Dawang Aboriginal Corporation (RDAC) in the East Kimberley. The Certificate II and Certificate III in Aquaculture training using Cherabin was originally provided to support skill development within the cohort to build capacity amongst participants prospective in gaining employment with Project Sea Dragon prawn farm but also to promote a unique indigenous business development opportunity that sought to integrate tourism components to assist in underwriting the total cost of operations.

A number of factors including inadequate supply of water to the site prevented meaningful and accountable training and assessment from continuing at the Mud Springs site and training ceased early 2018 and the project was suspended while a new partner was sourced.

Training for the current project commenced in November 2018 under the same structure as that delivered to RDAC but to students supplied through Emama Nguda Aboriginal Corporation (ENAC) using their developing aquaculture site. ENAC sourced these students through a local Community Development Program (CDP) hosted by Winun Ngari Employment Services who administered the local CDP programs on behalf of the federal government. Training was conducted at the ENAC site through 2018 and up to June 2019 when training was suspended again, due to operational difficulties within Winun Ngari Employment Services. These difficulties effectively ended the supply of students to ENAC. ENAC management managed to obtain funding to employ four full-time staff through late 2019 enabling the training to continue in 2020. Before commencement of training in 2020 COVID-19 events occurred preventing any training activities from occurring in any meaningful manner. Training finally recommenced through late 2020 and was currently in its final stages at the time of drafting this report.

Throughout the duration of the project BAC not only provided training and assessment supporting skills development to the project but also worked alongside ENAC to continuously assess hatchery rearing techniques providing a realistic benchmark for the hatchery phase, enabling improved supply of adequate numbers of cherabin Post Larvae (PL's) for partners to grow cherabin in their ponds, and enable assessment of on-farm production parameters which could be embedded into the development of tourism integrated business model.

2.0 Aquaculture methodologies

Throughout the project North Regional TAFE and Emama Nguda Aboriginal Corporation have worked to refine some benchmark culture processes to reliably support a developing Cherabin supply chain for Western Australian aquaculture operators. Anecdotal evidence, particularly observations during larval development support the idea that the cultured animals aren't *Macrobracium rosenbergii*. The current assumption is that the species is *Macrobrachium spinipes*, a species that appears to have different biological and growth characteristics that may prove to be more efficient than *M.rosenbergii*.

It is important to note that there were many challenges to overcome throughout the project. These are highlighted in section 7.0. Both NRTAFE and ENAC have steadily been developing systems and practices to overcome these challenges to produce reliable post larvae.

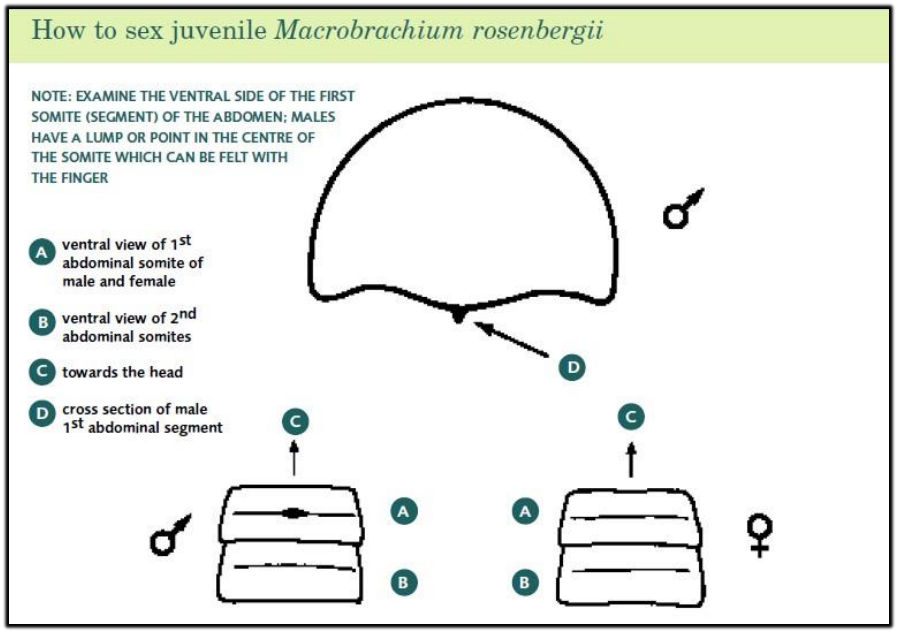
In addition to this final report a summary of larval culture systems and practices has been defined. This summary reflects the practices developed and used at NRTAFE to produce the most reliable batches of post larval cherabin throughout the project.

2.1 Broodstock selection and collection

Originally North Regional TAFE had planned to collect gravid female Cherabin sourced from the Fitzroy River with the aim of holding them at their aquaculture centre until spawning occurred and then culling the animals once they had hatched their larvae.

Late and inadequate rainfall events during consecutive 2017-18 and 2018 -19 wet seasons led to unreliable collection of gravid females from the Fitzroy River, WA. Additionally, the size of the animals throughout the broodstock collections made it difficult to determine the sex of many of the collected animals. A number of large male specimens were collected during one the collection events in proximity to Camballin. In-lieu of a lack of naturally gravid females these males were transported to the Broome aquaculture centre to test the viability of captive breeding.

Similar to *Macrobrachium rosenbergii*, females can be selected at capture location by checking the ventral side of the tail and checking for absence of lump on the second abdominal segment as shown in image 2.1.1. This technique was shown to be reliable in animals greater than 80mm in total length, however was unreliable in animals <80mm total length.



** Imaged extracted from FAO fisheries technical paper 428, page 7.

Image 2.1.1 – Sexing *Macrobrachium rosenbergii*

2.2 Broodstock spawning

A range of research documents indicate that approximately 1,000 larvae can be produced per gram per female.

BAC proposed that on average females could be 20 – 30g and therefore capable of producing 20,000 – 30,000 larvae. As describe in section 2.1 wild capture of berried females was difficult through the project initiating the use of captive breeding strategies. Breeding systems at BAC were maintained under ambient conditions however heaters were periodically used to ensure temperature fluctuations were minimised through coming into and out of the dry season. Systems at ENAC were maintained solely under ambient conditions.

Captive breeding with *M.spinipies* occurred at the NRTAFE and ENAC sites using slightly different culture systems designs. Both sites utilised existing equipment to construct their respective systems. Each of the culture systems utilised recirculating water mechanisms. NRTAFE utilised rectangular raceway style tanks while ENAC used a combination of flat and tapered round aquaculture tanks without any real apparent differences in spawning success. BAC's systems are described in greater detail in the document - a *summary of key hatchery processes for Macrobrachium spinipies.*

Table 2.2 – Estimated stocking density of broodstock

Site	System area	System stocking density	Sex ratio
ENAC	$34m^2$	$2/m^2$	3:1
NRTAFE	$12m^2$	$2/m^2$	3:1

Broodstock animals were fed a range of diets that differed between the BAC and ENAC sites. BAC did make some loose links to spawning success with changing diets. Anecdotal evidence suggests that the best performance from BAC broodstock appeared to occur where a combination of a commercially produced diet, as shown in Table 2.3, and the regular inclusion of thawed frozen bloodworms as a supplement. This theory was untested through the course of the project. BAC technical staff have commented that larval hatch success and survival diminished through periods where supply of bloodworms was limited or excluded. ENAC used CP shrimp feed 4005 which is a grower pellet for animals larger than 30g and the inclusion of bloodworms, sparingly, through the project, citing the costs of bloodworm as prohibitive. The use of thawed bloodworms could be seen to be comparatively costly when compared to that of compound pellet diets. The bloodworms also posed a biosecurity risk, as polychaete worms are a known carrier of the white spot syndrome virus which is known to affect Macrobrachium spp. Research by Nandlal, S and Pickering, T, 2005 from animals grown in Pacific Island countries shows that there may be a potential deficiency in the commercially produced diets used throughout the project to supply sufficient levels of fat.

Table 2.3 - Feed composition comparison

Site	Feed Supplier	% Protein	% Fat	% Fibre	% Moisture
BAC	**CP Feeds (B2)**	50	8	4	12
ENAC	**CP Feeds (4005)**	35	4	4	12
Nandlal & Pickering		40	10	8	-

*Protein and Fat Values are Min

*Fibre and Moisture values are Max

Ecdysis periods and spawning activity occurred without further stimulation around full and new moon periods at both the BAC and ENAC sites. No assessment of spawning success comparison relating to lunar phase was undertaken to compare differences between full and new moon periods. Males could be seen actively engaged in mating, pairing and guarding activity through these periods. These behaviours were easily recognisable by technical staff, enabling them to track egg laying and fertilisation of individuals.

Animals were left in the broodstock holding tanks and observed daily for egg retention until day 13 - 16 post fertilisation when eye spotting of eggs was observable. Once eyespots were observed the females were moved into larval rearing tanks. The berried females were held in custom built floating plastic mesh baskets within these tanks, facilitating easy removal of female broodstock post hatch. Extensive periods of holding in these baskets may have impacted hatching success especially where ineffective analysis of egg and eye spot development occurred.

The colour development in wild animal's transitions from orange to dull brown to a dark brown or brownish grey prior to eyespot development and like that of image 2.2 and extract from the document, 'breeding and larval rearing in hatchery of *Macrobrachium vollenhoveii* in a perspective of biological control against human Schistosomiasis in tropical area' (Ndao *et. al)*. It was initially proposed that captive bred animals would produce eggs of similar colour transitions / development however, this wasn't apparent across the BAC and ENAC sites.

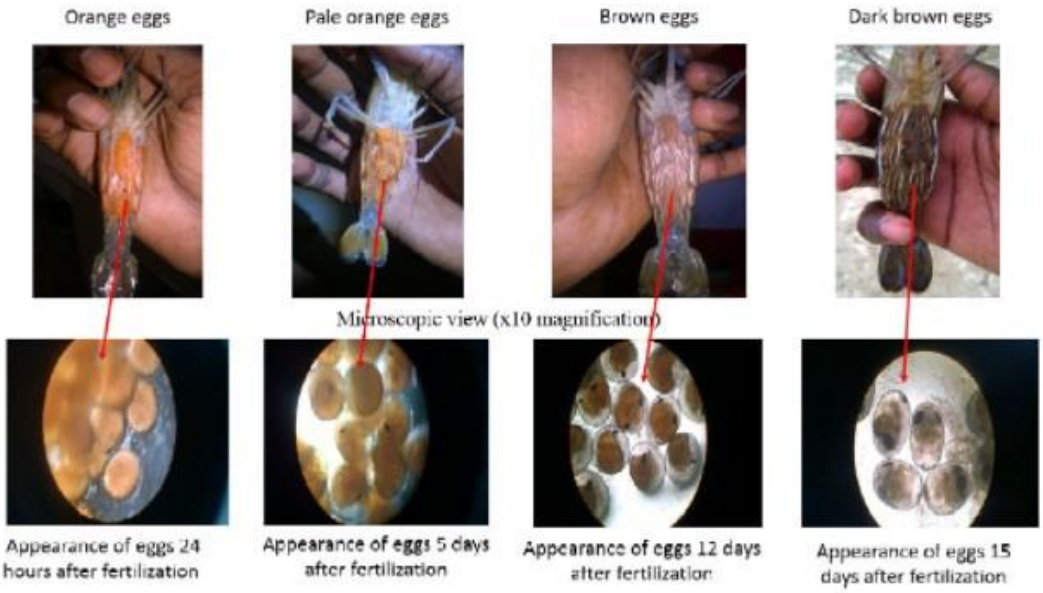
Differences in physical egg colouration between the BAC and ENAC sites were observed between participating groups but not formally documented. Fertilised eggs at BAC exhibited a range of pale colourations from orange through to bright green and on to dull green prior to eye spotting. Fertilised eggs from females held in ENAC recirculation systems more closely resembled the developmental colourations shown in Image 2.2 but the final stage more of a greyish brown, suggesting the variations within broodstock diets may have an impact on egg quality between the sites.

During a partial harvest of a pond the ENAC team recovered many berried females exhibiting further differences in egg colouration. The eggs recovered from the pond cultured females were a rich orange-brown colour prior to eye spotting and produced a very successful hatch once moved to the hatchery. It has been suggested that this rich colouration indicates premium egg nutrition and directly correlates to good levels of dietary cholesterol for the females prior to spawning, Teshima, s et al. 1997 showed evidence that cholesterol levels of 0.1% are an important supplement to juvenile freshwater prawns to achieve maximum growth. It is unclear what prey items were readily available

in the pond that may have provided adequate nutrition or whether dietary cholesterol played a role in producing this perceived improvement in egg quality.

Berried females collected from the pond were stocked into holding tanks in the ENAC hatchery, held in batches, and allowed to spawn naturally over a period of a few days. Hatching tanks were partially drained by siphoning to capture freshly hatched larvae.

Image 2.2 – Egg development of M.vollenhovenii – image extracted from Papa Demba Ndao et. al



It was common to see mortality in large (dominant) males through unidentified mechanisms, especially leading into or out of spawning season (wet season). Speculation exists around the expected natural age at mortality for the males at approximately 2 – 3 years of age. Cannibalism, normally during Ecdysis, in breeding tanks existed where numbers of animals were unaccounted for, and where numbers were found to exceed 2/m^2.

2.3 Larval Culture

Literature widely documents fecundity amongst *M.Rosenbergii* at 1,000 eggs/g and it had been expected that the project would be able to collect berried females from the wild and produce approximately this amount, however no success in collecting berried females occurred. Actual fecundity of captive bred females from either ENAC or BAC sites was never measured preventing a comparison to be made. Hatch rates were routinely measured once hatching was completed and have been compared in Table 2.3 against researched optimum densities of >40 Larvae/L.

Table 2.3 – Comparison of actual stocking rates 2019 - 2021

Year	Min. #'s Stocking	Max #'s Stocking	Mean #'s stocking	Mean stocking Density
2019	2,750	7,480	4,600	23/L
2020	600	7,480	2,577	13/L
2021	240	12,000*	3,483	17/L
Recommended Stocking density				>40/L

* two females contributed to this spawn

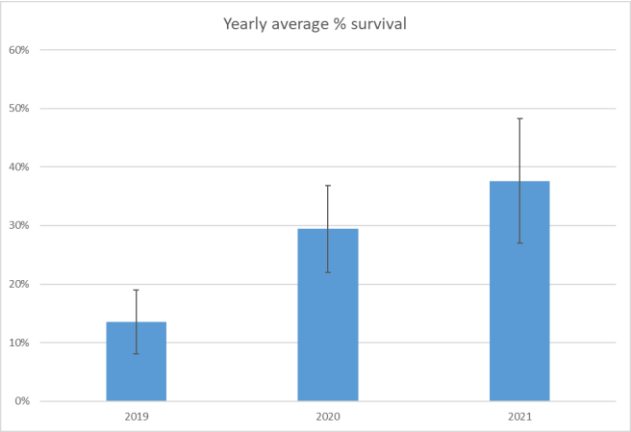
Throughout the project processes and culture systems support larval development changed to ensure the unforeseen and unpredicted shortfalls in apparent larval hatch rates of female breeders could be overcome at the BAC site. This hindered the progress of truly replicated trials of stocking densities and feeding regimes to identify optimised recommendations.

Larval survival was variable throughout the project and likely linked to the learning and challenging experiences of the BAC team over the course of the project including,

- Biosecurity and sanitation of systems
- Biosecurity of live food including algae
- Larval nutrition
- Broodstock nutrition
- Larval development stages

Figure 2.3 depicts improved larval survival through 2019 – 2021 until harvest to nursery. A final account and description of the system and larval process that worked to improve larval survival and produce reliable numbers of PL's from BAC is described in the document - a *summary of key hatchery processes for Macrobrachium spinipies.*

Figure 2.3 – Yearly survival comparison 2019 - 2021



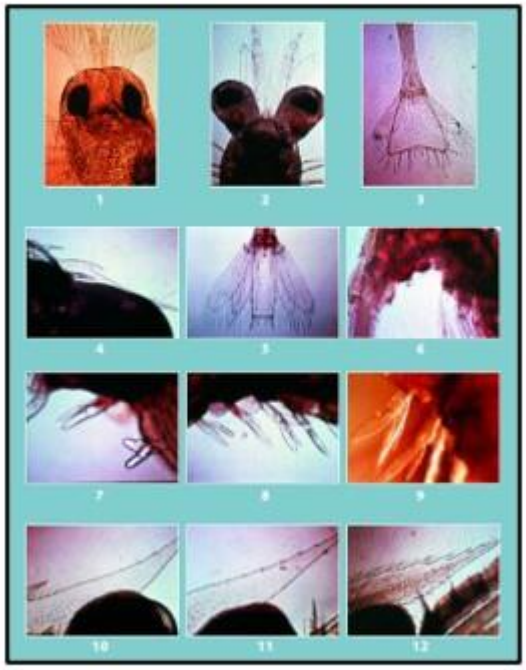
The Emama Nguda aquaculture site has no current immediate access to salt or brackish water and an operational constraint on the team's ability to achieve truly successful larval rearing outcomes. Larvae were sourced from their own collected and reared breeders and cultured in freshwater with minimal survival through to post larvae.

Early larval mass mortality occurred at both the BAC and ENAC sites. The mechanisms contributing to this are poorly understood and were previously theorised to be linked to poor nutrition within brood animals or the larvae however, Alam S et.al described two potential major causes of mortality as pathogenic in nature and have identified nodavirus (MrNV) and Vibrio sp. as the most likely contributors to mortality.

Testing of water sources in the cherabin systems showed that there was a significant presence of Vibrio sp. on TCBS agar plates after 24hr incubation at ambient temperature. Testing highlighted and absence of Vibrio's in the breeding tanks and their presence in the larval and post larval systems. Subsequent testing of Live food and Algae also highlighted their presence.

Larval development assessment was difficult to completely assess as the developing animals didn't replicate that of *M.rosenbergii* as shown in Image 2.3 – *Macrobrachium rosenbergii* larval development. The detail relating to the differences wasn't really quantified until late in the project when it was apparent the *M.spinipies* developmental stages were slightly different. Work to classify morphological developmental stages for *M.spinipies* was being conducted to solidify actual descriptions but the onset of the dry season 2021 prevented this from occurring prior to the project conclusion.

Image – 2.3 - *Macrobrachium rosenbergii* larval development



*Extracted from FAO, Annex 1, page 146 – Source; Takuji Fujimura

Image – 2.3.1 - Modified larval development reflecting Macrobrachium spinipies stages

*Image modified from FAO, Annex 1, page 146 – Source; Takuji Fujimura

It appeared to be that the most noticeable difference occurred with stage five of development as documented in image 2.3 when the Telson begins to lengthen and narrow. In *M.spinipies* this development appeared to occur after the full range of pleopod development at stage nine as shown in image 2.3.1 instead of occurring at stage five.

Larval nutrition was a challenge in the beginning, the use of egg custard feeds to directly feed or indirectly feed larvae via live feed enrichment was a fundamental failure. These feeds were prepared on site at BAC. Feed 1 (appendix 1.1) proved to be unreliable in its consistency and difficulty in being able to push through a sieve and Feed 2 (appendix 1.2) as detailed by Kovalenko E.E *et al. (2002)* was trialled but inconsistencies and in the product post autoclaving and a lack of available vitamin premixes at the time made it difficult to continue with the diet.

In early 2019 it was identified that a green-water larval management approach adapted from Lober, M and Zeng, C (2009) where they assessed microalgae concentrations on larval survival,

development and growth of an Australian strain of *M.rosenbergii* and found that larval survival to PL was highest at algae cell (*Nanochloropsis* sp.) densities higher than 12.5 x 10^5 cells/ml.

Although there were some encouraging metamorphosis results for batches throughout 2019-20, the BAC team felt that the PL process was a potential bottle neck in a hatchery's ability to produce viable numbers of PL's to farm, citing that potential cannibalistic activity was decreasing the total survival to PL.

2.4 Post larval development

Several cherabin batches at post larval (PL's) stage were reared in a small recirculating system at BAC with extremely variable survival results. The system parameters and design are described in the document - *A summary of key hatchery processes for Macrobrachium spinipies.* Animals were stocked into the system and acclimated to zero ppt salinity over 3 – 5 days. incrementally increasing the tank volumes with the inclusion of freshwater until zero ppt was achieved.

No distinct assessment of the impact of stocking density on survival was undertaken due to this variability. It is believed that the variability in success could be primarily attributed to broodstock and egg nutrition which affected ongoing developmental success with the animals. Additionally, an incomplete understanding of the animals weaning requirements and time to translocation may also contributed to the variability in the survival. This potentially led to unconfirmed mortalities predicted to be cannibalism, as an absence of visible mortality was common. Post larval culture periods exceeding two weeks showed a rapid decline in animal and again predicted to be linked to cannibalistic activity.

Delays in translocation of animals from BAC to ENAC occurred through the project ultimately reducing survival and ability to supply adequate numbers of PL's. These delays existed due to barriers with biosecurity regulators making animal despatch difficult to coordinate in a timely manner. These delays saw animals held over periods long than predicted and led to increased apparent cannibalistic activity.

Comprehensive investigation for post larval growth performance – nursery phase, wasn't conducted as it was proposed that the juvenile prawns would be moved to ponds early in the juvenile stage.

2.5 Pond stocking

While BAC and ENAC managed to improve survival of larval cherabin to the juvenile stage, post larval survival rates using tank culture were lower than anticipated. The reduced numbers of juveniles limited the ability for the project to productively assess the pond phase. The impact of this was not clear until the time of writing this document and therefore some of the outcomes for this milestone aren't very well defined and directly linked to lower than predicted stocking rates of ponds.

A fair degree of conflict relating to pond stocking rates exists within current literature. FAO documentation for *M.Rosenbergii* indicates the rate of stocking is variable based on size/age at stocking but identified a stocking rate of 200 Juveniles/m^2 can be used with animals in the size range produced from BAC. Mente, E (2008) research indicates that a peak stocking rate of 20 juveniles/m^2 can be utilised to achieve effective production outcomes, however it is believed that this rate may

fall below that to facilitate best cost of production. In their research of density dependant factors relating to growth and production of *M.Rosenbergii*, Paul, P et.al showed that stocking rates of 40 PL's/m^2 achieved the best growth, FCR and survival between test ponds stocked at 40,50 and 80 PL's/m^2. The value of 40PL's/m^2 falls within the observed range of acceptable stocking rates for Black Tiger Prawns of 20 – 60PL's/m^2. 40PL's /m^2 could be considered a realistic starting point for intensive culture of *M.Spinipies*, possibly enabling farmers to meet or exceed financial expectations on costs of production.

Regardless of the documented ideal stocking rates from cited literature, a fundamental constraint on the projects ability to produce ideal numbers of PL's for pond stocking resided with the ability of the project to produce enough PL's (as described in section 2.3). The predicted hatchery output was initially proposed to be approximately 20,000 PL's per batch. This was never achieved.

The ability for the project to produce, consistent and ideal volume of PL's to stock at the estimated rate of 20 PL's/m^2 resulted in low pond stocking densities. The stocked rates observed were representative of those used for extensive (low input) culture of *M.Rosenbergii*, stocked at rates of ~1PL/m^2 as described by. ENAC stocked pond 1 sequentially over a period with small batches of animals and due to this, the pond was run using extensive culture mechanisms. The pond was allowed to naturally mature, and no control of weeds or other competitive insects occurred. No comment can be made on any final production outcomes as reliable measurement of commercial growth as the trial was compromised by the ongoing stocking making it difficult to ascertain commercially representative production parameters.

A second pond (Pond 2) was constructed through 2020. The natural aging of the pond differed from that of Pond 1 and has remained at significantly higher turbidity and possibly linked to the addition of water soon after construction completion and the use of an aspirator aerator which may have prevented suspended particles from settling. No fertilisation of this pond occurred and as such no algae bloom or additional weed growth has been observed. Pond 2 was stocked with an estimated 3,000 PL's (3 PL's/m^2) during 2021 and aligned with Akter,S et al. description of improved-extensive culture. While the stocking rate is still lower than the expected ideal of 40PL's/m^2 it represents improvement towards semi-intensive culture practices and does enable more reliable analysis of commercial production modelling to be undertaken.

At time of PL liberation to Pond 2 the water temperature decreased rapidly with the onset of the dry season and animal activity has been observed as low and growth expected to be low. At the time of writing Pond 2 hadn't been harvested enabling a good assessment of production outcomes to be undertaken.

An interim and partial harvest of pond one did occur with the aim of checking for health and growth performance and highlighted high variability in size that has been suggested to occur from growth differences between male and female stock at time of assessment. It should be commented that feeding trays and interim assessment of animal performance from either of the ponds with traps and nets yielded little result making any interim assessment of growth difficult. It is suggested that the low stocking rates within each of the ponds may have been a critical factor in failure to capture animals for interim sampling to occur. Additionally, the logistics in draining ponds based on their basic design also made it difficult to easily drain a pond to undertake any assessment.

Table 2.5 – Stocking of ponds at ENAC

Pond Number	Number of PL's / m^2	Type of Culture	Comment
1	1	Extensive	Pond stocked sequentially with small batches compromising any batch analysis of production performance. Partial harvest yielded heterogenous growth.
2	3	Improved Extensive	Pond stocked 2021 with a combined batch from BAC And ENAC, however it wasn't harvested before the end of the project duration.

2.6 Training Engagement

Through 2019 re-negotiation of training arrangements were undertaken for the current project. ENAC was able to provide four students through their aquaculture operation enabling the project to continue the path of its origin. ENAC had an appetite to have fully autonomous workers at the end of the training program, hence the program was established at the Certificate III level and structured to include five one-week tutorial blocks to boost learner confidence facilitating higher levels of completion.

Table 2.6.1 outlines the training schedule as it was delivered, this may differ slightly from the initial plan as a number of training blocks were postponed due to unforeseen operational and external factors E.g., Sick leave. Students are currently started but not finalised for blocks 9 – 11. Additional information on the training outcomes described in section 5.2.

Table 2.6.1 - Training Program 2020 - 2021

BLOCK	LOCATION	DATE	CONTENT
1	Derby	July 2020	SAFETY FOR AQUACULTURE PROJECTS
2	Derby	August 2020	USIQ TUTORIAL - MAINTAINING WATER QUALITY AND SYSTEMS TUTORIAL
3	Derby	September 2020	USIQ TUTORIAL - MAINTAINING WATER QUALITY AND SYSTEMS TUTORIAL
4	Broome	November 2020	USIQ TUTORIAL - MAINTAINING WATER QUALITY AND SYSTEMS TUTORIAL
5	Derby	January 2021	IMPROVING SYSTEMS FOR HYGIENE AND DISEASE
6	Broome	February 2021	ESSENTIAL HATCHERY SUPPORT
7	Broome	March 2021	USIQ TUTORIAL – FACILITATING TEAMWORK FOR PRODUCTION GOALS

8	Derby	April 2021	INSTALLATION OR CONSTRUCTION OF FARM STRUCTURES
9	Broome	In progress	UNDERTAKING SUSTAINABLE FARMING PRACTICES
10	Derby	In progress	MONITORING AND MAINTAINING HEALTHY STOCK
11	Derby	In progress	ESTABLISHING STOCK TRANSPORT MECHANISMS

During the tutorials the students participated in contextualised subject content relating to;

- Self-awareness and performance development
- Teamwork supporting group performance
- Problem solving and concept development
- Personal Initiatives
- Speaking and Listening and communication

Through this process the lecturer was able to identify the difference in the pre-existing capabilities of each student with the intent of focussed learning and support for those in most need. The flexible nature of the tutorial program enables a strengths / weakness approach to be utilised where we able to focus on what the learner can do confidently to increase confidence, to tackle more challenging tasks. Most of the content was delivered in a practical context where students were encouraged to actively participate in teamwork tasks to achieve outcomes for the ENAC site but within their own strengths.

3.0 Business development

It is important to note that ENAC were again unaware of the requirements within this milestone and as such they engaged their own practices to form strategies relating to the development of their business in culturing cherabin. In this process it can be commented that their focus aligns with the original intent of the project by integrating tourism as a major source of income to offset the specific costs of culturing the cherabin. They believe that they can adopt a model like that of Huka Park Prawns (New Zealand). ENAC have commented that their approaches to developing their business are specific to their needs and may not reflect that of other interested businesses or indigenous enterprises.

3.1 Local product development

The simple email engagement methodologies used to canvas potential local demand weren't successful in getting any feedback. Fervor Foods are a WA travelling restaurant that use locally sourced seasonal products on their menu including the use of bushfoods and those foods with good provenance or storytelling. Their owner operator has raised great interest in trialling the products to assess suitability for inclusion on their menu.

More consolidated information is documented in the attached summary, 'A Cherabin business development summary'.

3.2 Collaborative development of key information

A simplified business development methodology has been completed and available in the document "A Cherabin Business Development Model" which may assist businesses with interest in cherabin to assess their ability to grow Cherabin. Additionally, the document - *a summary of key hatchery processes for Macrobrachium spinipies* will help them to better understand the culture processes of larval cherabin, and assess their need to purchase or grow their own.

4.0 Stakeholder engagement

More complete and accurate detail around stakeholder engagement can be found in CRC NA-Cherabin Project - Quarter 4 - Communication plan.

5.0 Key Outcomes

5.1 Production outcomes

Production outcomes throughout the project were extremely variable and largely attributed to previously unidentified production requirements for *M. spinipies* prior to the commencement of the project. A great deal of effort was invested in refining components of the larval production process to achieve a reasonable level of repeatability in the larval culture process. An account of the key production processes and systems throughout the larval culture cycle is given in the document - *a summary of key hatchery processes for Macrobrachium spinipies.*

Viable production outcomes throughout the project were theorised to be associated with the amount of time allocated to the maintenance of the Cherabin broodstock and larvae rearing systems. Throughout 2020 and 2021 this theory was evaluated by allocating more time to the husbandry of breeding, larval, and post larval animals. Additionally, ongoing exposure of all staff members to contemporary husbandry practices, including additional biosecurity controls allowed for better continuity and coordination of daily or scheduled tasks.

Broodstock, larval nutritional management, and stage of PL at harvest to nursery have been proposed as a critical factors to maximise larval. Some of the key improvements to offset the range of challenges are defined in the following sections and worked to produce the single biggest post larval output in March 2021 of approximately 1,500 animals at PL15. A further batch successful larval batch of approximately 10,000 made it through to metamorphosis but an unprecedented cold weather event saw this batch suffer complete mortality shortly after metamorphosis.

5.1.1 – Broodstock Nutrition

It is still not completely understood what the requirements are to facilitate the best quality outcomes for breeding animals it is suggested that the supplementation of pelletised feeds with bloodworms (polychaete worms) may produce more stable and reliably healthy batches larvae. Bischoff, A 2014, comments that "polychaete's can be used to induce a controlled spawning within

several fish and crustacean species and are able to supply spawners with essential nutrients such as fatty acids and amino acids". It may be that the diets used by BAC and ENAC in this project have been deficient in fatty and amino acids and the addition of blood worms has helped to bridge a nutritional gap.

The recovery of premium quality eggs from the ponds at ENAC highlights a potential efficiency in the nutritional management of broodstock by enabling the animals to feed on naturally available prey items without the need for establishing tank-based breeding systems that may not produce the best larval rearing outcomes.

In lieu of needing to condition breeding animals using tank-based systems the inclusion of bloodworms is recommended to fill the apparent fat deficiency observed between the commercial CP feeds and that recommended by Nandlal, S and Pickering, T, 2005. BAC fed pelletised fed to the breeding animals once per day in the mornings at the rate of 2% of tanks biomass. The most reliable feeding strategy incorporated supplemental feeding of bloodworms at 4% biomass three days in any week as shown in Table 5.1 – Suggested broodstock feeding schedule. Bloodworms were thawed and cut into pieces allowing for even distribution to all breeding animals and fed alongside pellets immediately when thawed.

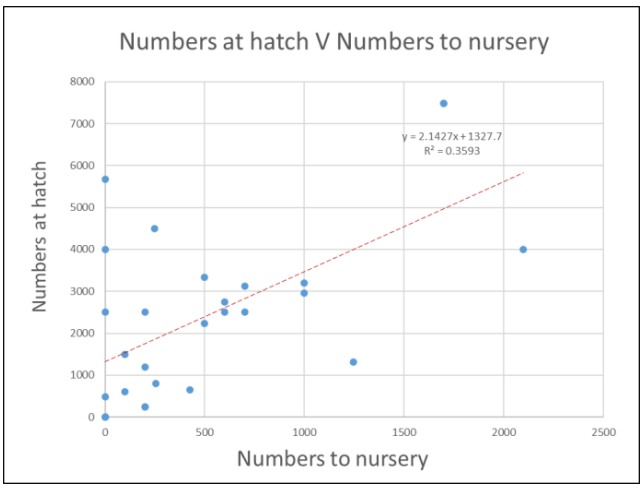
Table 5.1 - Suggested Broodstock Feeding Schedule

Day	Mon	Tue	Wed	Thu	Fri	Sat	Sun
Feed	4g Pellets	4g Pellets 8g B/worm	4g Pellets	4g Pellets 8g B/worm	4g Pellets	4g Pellets 8g B/worm	4g Pellets

* Feeding schedule based on a total biomass of 200g

We assume that improved egg quality is directly linked to the success of a batch at metamorphosis. This is demonstrated in Figure 5.1 – egg health dependant survival analysis, where a positive correlation between numbers of animals at hatch and survival to nursery is evident. However, it can't be excluded that the level of staff learning throughout the project hasn't compromised this assumption and more repeated and reliable research should be conducted around the effect of broodstock nutrition on the larval rearing process.

Figure 5.1 – Egg health dependant survival analysis



5.1.2 – Larval Nutrition

The green-water larval culture as described by Lober.M and Zeng.C (2009) was used to as a general guide for the BAC to structure a regime that fit within the limitations of their site. An example of a finalised larval regime that had some reliability to produce larvae is described in Appendix 2.0. In this regime it is assumed that artemia numbers would be managed on a volume basis and not the numbers of animals within the tank. This was due to potential inaccuracies in the counting methodologies for the larval cherabin.

Freshly hatched, sanitized and cleaned Artemia nauplii were added to the 200l each morning at the rate of 5 Artemia/ml for stage 0 – 2, 10 Artemia/ml for stage 3-5. Stage 6 – 10 were fed sanitized and cleaned enriched day old Artemia at 10 artemia/ml. The day old Artemia were enriched for approximately one hour in a 5L volumetric jug using 2/ml of Iso-1800 instant algae (Reed Mariculture). Artemia densities were estimated throughout the day using a 10ml glass pipette, and an additional feed of artemia added around 3pm ensuring densities were maintained at approximately 10 Artemia/ml.

Weaning of the cherabin larvae was undertaken using CP Feeds weaning diet, TNT 128. The feed was added sparingly to culture tanks between live feeds at roughly 11am and 1pm.

The addition of Nanno-3800 instant algae (Reed Mariculture) ceased through 2020 and 2021 after it was identified to potentially contribute to disease concerns and Iso-1800 used in its place. The Iso-1800 was added to the 200l tank morning and afternoon alongside Artemia. 0.5ml of the algae was suspended in a 2l measuring jug and then directly pouring it over the air to aid rapid mixing and prevent settling. The use of a low algae concentration contravenes Lober.M and Zeng.C (2009) research but deemed necessary as settling of instant algae can contribute to degradation of water quality.

While this Larval nutritional strategy worked to produce reliable survival to PL it is believed that improvements can be made using live algae, especially if batched algal culture techniques are likely to be used. As it may enable better biosecurity control and available nutrition to occur while assisting to mitigate risks with water quality at higher densities of algae. Additionally, the inclusion of live diatom algae may provide additional improvements to growth and survival, Lal, M. *et al*. (2014) utilised naturally occurring diatoms of genera *Nitzschia*, *Navicula* and *Skeletonema* as seedstocks in their assessment of larval rearing of Macrobrachium lar. Thalassiosira sp. or similar are known to exist in Australian estuarine environments and should be considered as alternatives to marine diatoms. In-lieu of not having a commercially functioning algae lab businesses should consider the controlled use of instant algae.

5.1.3 – Biosecurity

After the identification of the presence of Vibrio sp. in the Cherabin larval rearing, Post Larval nursery tanks and feed sources it was determined that systems would be periodically shut down and sanitised before being re-stablished. Each of the tanks was independently cleaned with detergent, rinsed with freshwater before being scrubbed with a 1,000ppm solution of sodium hypochlorite. Each of the static tanks in the larval rearing system were then filled to overflow with freshwater and a 150ppm solution of sodium hypochlorite left to stand overnight before dumping. The post larval system was filled to overflow with clean saltwater and 150ppm sodium hypochlorite added to the system which was allowed to circulate for approximately one hour before being switched off and let stand overnight before dumping. Both systems were dried out for a period of a week prior to recommencing any work.

Bacterial problems with bottled instant algae were ruled out by excluding the bottles that returned positive tests on TCBS agar plates. This meant the complete elimination of Nannochloropsis (Bottle 1) and replacing them 1:1 with Isochrysis (Bottle 2).

Due to the lack of bacteria in the breeding systems no quarantining or prophylactic treatment of the eggs and females was deemed unnecessary at the time but closer assessment of eggs and females should be conducted if the presence of pathogens is detected and the source isn't confirmed.

Image 5.0 – Instant Algae used in larval rearing

Bottle 1 **Bottle 2**

Biosecurity concerns were also identified with live feed (artemia) and a sanitation protocol was set in place to ensure cross-contamination was minimised. All hatching tanks were scrubbed with detergent and rinsed and then chlorinated prior to and after use using a concentrated chlorine scrub (1,000ppm), all other equipment was soaked in a bath of 1,000 ppm sodium hypochlorite.

Additionally, hatched, and harvested artemia were sanitised for a minimum of 30min in a 50ppm solution of formalin and seawater. The artemia were then rinsed with clean saltwater before being added to the larval culture tanks at the scheduled feed rate.

Staff and students were encouraged to be diligent in the chlorination of all equipment used in the larval rearing process and chlorine baths were regularly monitored for hygiene and changed as required.

While not solely responsible for improvements to larval survival the improvements in hygienic heightened biosecurity awareness and as such processes were better defined, documented and implemented within the hatchery.

5.1.4 – Larval harvest, counting and timing.

A really exciting outcome from the project was that the growth and development of *M.spinipies* was apparently faster than that of other cultured species as shown in Table 5.1.1. Estimates from animals grown at the BAC hatchery consistently show that PL stage is achievable in approximately 12 days. It was difficult to assess and complete a full descriptive analysis of how long the PL process took as many of the batches were low in number and collecting animals was time consuming. Most evidence supporting the time to PL relied on visual observation of the animals to form benthic behaviours and upright swimming rather than microscopic observation of morphological features.

Table 5.1.1 - Optimal growth comparison of cultured *Macrobrachium* sp.

	Species	Location	Days as Larvae	Stages to metamorphosis
1	*M.spinipies*	Australia	12*	10
2	*M.rosenbergii* [a]	Australia	30	11
3	*M.rosenbergii*	SE. Asia	19	11
4	*M.equidens*	Brasil	23	10
5	*M.vollenhovenii*	Senegal	31	15

* Animals moved prior to metamorphosis, benthic behaviour observed
[a] Identifed as the Australian strain
^ Information sourced from, 2 Lober, M. & Zeng, C (2009),3 Nik Sin, N.N & Shapawi, R (2017), 4 (Gomes, J.N, 2013), 5 (Ndao,P.D, 2019)

This rapid growth performance is a potential indicator that the overall growth performance of the animals is also faster than that of other species and possibly more efficient. This also reduces risks within larval rearing failure by actively reducing associated biosecurity and water quality risks within any batch, but also minimises the available time to make technical mistakes.

To overcome the suspected cannibalistic activity during metamorphosis the BAC team commenced early harvesting of larvae during 2020. The animals were moved from larval culture tanks and into 900L nursery tanks with suspended substrates added to each tank, increasing the total surface area of each tank. The early harvest of larvae prior to metamorphosis assumed that increasing available surface area should enable individual prawn's greater access to space to undertake the moult, reduce cannibalism and competitive stresses. The success of this approach to improving survival is shown in figure 5. 1.1 – Survival comparison at transition to PL where survival is demonstrated to improve by 100% and is one of the most significant outcomes for this life stage for M.spinipies culture.

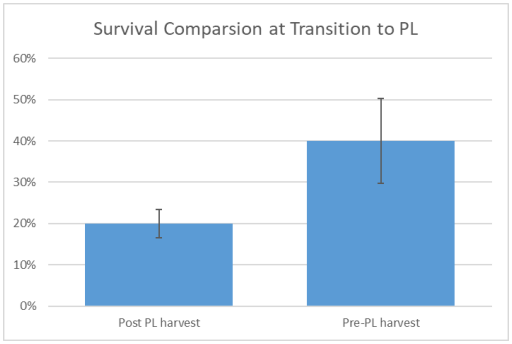


Figure 5.1.1 – Survival comparison at transition to PL

5.1.5 – Post larval processes

The project was limited in its ability to clearly replicate and refine post larval culture processes. This was largely linked to the challenges associated with producing enough larvae to stock into juvenile rearing tanks. More information on this is described in a summary of key hatchery processes for Macrobrachium *spinipies*. The single biggest outcome from the project relating to this stage of development was the need to harvest juveniles within 14 days of metamorphosis. A significant decrease in survival was noted for time frames longer than 14 days however, this observation wasn't challenged with robust testing but may be linked to a lack of available space or some inadequacies in post-larval nutrition.

While it has been theorised by the BAC team that the best approach to minimising post-larval mortality is to stock the juvenile prawns into ponds at day 10 – 14 the pond-based phase of the project highlighted that his might negatively impact the survival of the animals post stocking, and proposed that holding PL's for extended periods in the nursery phase maybe required to improve survival in grow out.

5.2 Training

Student retention and attendance throughout the training program was 100%. Throughout the training program key components of cherabin contemporary hatchery and nursery processes were utilised to enable the students to learn skills directly relevant to cherabin production. At times the students engaged in learning activities that were influential in managing challenges affecting larval survival within the Broome Aquaculture Centre cherabin systems. Some of these learning experiences have been adopted by NRTAFE and likely to be used within any future breeding work with the species.

A simplistic summary of the training program delivered over 2020 and 2021 is shown in Table 5.2.1 including the tutorial sessions. The integration of the USIQ tutorial programs was a defining success. Essentially the tutorial program is a way of expanding the learning journey without any required assessment. The tutorials enabled the students to take the time to learn critical underpinning skills

and gain better insights into the daily operations without the frustrations and fears associated with assessment, leaving the students better prepared for weeks of training and assessment.

Three of the four participants have been successful in meeting competency standards and have completed their Certificate III in Aquaculture. The fourth student has been able to demonstrate some competence but discontinued training due to other job prospects.

Table 5.2.1 – Summary of training delivery

BLOCK	CONTENT	Status	Comments
1	SAFETY FOR AQUACULTURE PROJECTS	Complete	Students successfully applied skills to assess potential and actual hazards relating to the worksites at ENAC and BAC.
4	USIQ TUTORIAL - MAINTAINING WATER QUALITY AND SYSTEMS TUTORIAL	Complete	Students work on building their core skills including, water quality monitoring, documenting critical parameters, understanding and designing standard operating procedures, participating and communicating in team meetings.
5	IMPROVING SYSTEMS FOR HYGIENE AND DISEASE	Complete	Students successfully applied skills relating to improving biosecurity processes with the whole farm including, quarantining, chemical sanitation of equipment, identification of key risks within the production environment.
6	ESSENTIAL HATCHERY SUPPORT	Complete	Students successfully applied skills relating to the management of cherabin broodstock, larval animals and live foods within the Broome Hatchery.
7	USIQ TUTORIAL – FACILITATING TEAMWORK FOR PRODUCTION GOALS	Complete	Students participated in activities to improve work performance of teams, including setting, reviewing and reflecting on personal work goals aligning with the needs of production goals and that of the team.
8	INSTALLATION OR CONSTRUCTION OF FARM STRUCTURES	Complete	Students successfully applied skills relating to the construction and installation of simple farming structures. The group used some plans and skills to build small PVC hides for juvenile cherabin.
9	ESTABLISHING STOCK TRANSPORT MECHANISMS	Complete	Students have been working on skills enabling them to transport live animals including cherabin typically using simple mechanisms. The group is yet to be assessed.
10	UNDERTAKING SUSTAINABLE FARMING PRACTICES	Complete	Students are currently working on developing their skills relating to addressing risks with resource usage, biosecurity and pest management.

11	MONITORING AND MAINTAINING HEALTHY STOCK	Complete	Students currently working on skills relating to the observation and recording of observations that may indicate ill health as well as identifying mechanisms that may work for the ENAC business to control and contain transmission of disease

5.3 Business model development

Due to a lack of clarity around the context of this component the project partners were unaware of whom should have undertaken this work. ENAC had already been working to build on pre-existing information obtained from market research prior them partnering with NRTAFE. They have cited that this information is linked to their refined strategies and they are pursuing their goals associated with these strategies.

North Regional TAFE outsourced market research to Honey and Fox in order to gain a better perspective of consumer and market profiling of the product or similar products. The information from this body of work establishes some key information around.

- Anticipated pricing of products based on international consumption
- Suggestions around what domestic wholesalers and consumers might need to build confidence in product
- Consumer profiling for targeted product development

In addition to this work from Honey and Fox ENAC supplied some information taken from their market research which aligns with that produced by Honey and Fox. More consolidated information is documented in the attached summary, 'A Cherabin business development summary'.

5.4 Stakeholder engagement

Some confusion about project milestones occurred during the project limited the ability for BAC and ENAC to coordinate representative pond trials, required data collection around the on-farm production parameters. ENAC and BAC agreed that no delegation or effective discussion about the management of pond trials occurred and as such representative production data wasn't confirmed.

More complete and accurate detail around stakeholder engagement can be found in CRC NA- Cherabin Project - Quarter 4 - Communication plan.

5.5 Related Documentation

The following documents are directly referred to and relevant to the project outcomes.

- A summary of key hatchery processes for Macrobrachium spinipies
- CRC NA- Cherabin Project - Quarter 3 - Milestone 29 and 30 brief
- CRC NA- Cherabin Project - Quarter 4 - Communication plan

- An integrated business development model for the Australian Cherabin

6.0 Project summary

The training component of the project was an ambitious milestone it was achieved and successful across multiple levels including,

- Testing a superior more supported training environment using additional non-assessable tutorial modules.
- Using real time farm and hatchery-based activities to achieve learning and pilot scale production outcomes.

While the training outcomes for the project were achieved, the described model isn't a recommended approach for learners who are new to the aquaculture industry or those participating in vocational learning for the first time. Instead, it is suggested that learning programs associated with aquaculture should use a scaffolded learning program that follows a strategy that,

1. Uses Gaining Access to Training and Employment (GATE) qualifications as the engagement program (entry point) to allow learners to familiarise themselves with the learning process in the aquaculture context.
2. Uses integrated tutorials, especially where Learning, Literacy and Numeracy barriers exist in learning groups.
3. Builds employment capacity with ongoing aquaculture skills development using the Certificate II qualification.
4. Finally, the use of the Certificate III qualification will give learners the opportunity to achieve greater levels of work autonomy

Using a scaffolded learning program over a period of 18 – 24 months, it is theorised that learners will have improved ability to enter the aquaculture industry and employers will have greater confidence that new recruits have an appetite to succeed in their aquaculture career.

The successes in larval rearing as described in section 5.1 are recommended for any operator as a starting point and are additionally documented in a summary of key hatchery processes for Macrobrachium spinipies, Table 6.1 – Key operational recommendations for Cherabin Hatcheries summarises critical points to focus on when considering business propositions and defining likely operational documentation, system design and overall production capabilities.

Table 6.1 – Key operational recommendations for Cherabin hatcheries.

Stage / Location	Need	Description	Action
Broodstock	Improved egg quality	Broodstock nutrition	Ensure bloodworms or another source fresh fatty and amino acids are routinely included as supplement to pelletised feeds

Broodstock	Improved egg quality	Broodstock nutrition	Integrate the use of breeders recovered from low density extensive ponds as regular source of fertile eggs for production plans
Broodstock	Ongoing stock management	Natural mortality	Ensure an annual collection of animals is undertaken from the wild or stocked ponds to overcome mortality in dominant males
Broodstock	Ongoing stock management	Minimise Cannibalism	Ensure stocking rates in breeding tanks don't exceed 2 animals / m^2
Broodstock	Improved hatching success	Stress management	Define stages of egg development early and ensure berried females aren't held for extensive periods in larval rearing tanks.
Broodstock	Improved overall performance	Broodstock domestication	Seek academic support to define domestication parameters for ongoing domestication program assisting with growth, disease resilience, cannibalism mitigation
Larvae	Improved larval capacity	System design	Seek support to design appropriate system scale to support predicted production cycles.
Larvae	Improved larval survival	Biosecurity	Integrate biosecurity monitoring as part of routine practice
Larvae	Improved larval survival	Biosecurity	Explore need and implications of in-situ sanitation of system equipment and live feeds
Larvae	Improved larval survival	Nutrition	Ensure adequate levels of live feeds are maintained, attempt to include live algae. Use instant algae (Reed Mariculture); or combinations of Iso-1800, Shellfish Diet 1800 and TW 1800.
Larvae	Improved Water Quality	Water Exchanges	Ensure twice daily water exchanges are undertaken from the bottom of larvae tanks and mortalities are counted and removed.
Post-Larvae	Improved survival at Metamorphosis	Minimise Cannibalism	Ensure stages of larval development are clearly defined and a strategy to move animals prior to metamorphosis, stage ten, is established.
Post-Larvae	Improved harvest biomass	Minimise Cannibalism	Introduce the use of easy to clean and install substrates to increase available

			space where large numbers of PL's are grown
Post-Larvae	Improved harvest biomass	Minimise Cannibalism	Ensure PL's are transported to ponds between day 10 – 14.
Hatchery	Improved production capabilities	Minimise Cannibalism and competitive behaviour	Seek to establish early identification of males and females assisting in single sex culture. Seek to use existing technology for genetic manipulation of animals for single sex culture.
Hatchery	Improved batch performance	Maximise HR inputs	Ensure enough time is allocated to the hatchery work to ensure sufficient and accountable work is being undertaken.
Hatchery	Improved batch performance	Minimise heterogenous growth	Refine grading techniques for larvae or early juveniles to allow cohort analysis to take place for percentage abundance of males and females.
Hatchery	Improved production planning	Controlled broodstock maturation	Refine mechanisms to support or overcome non-spawning periods during colder months.
Ponds	Improved egg quality	Broodstock nutrition	Integrate monitoring of breeding pond around new and full moon cycles for egg development

While these recommendations are a great starting point in managing the challenges identified through this project, they may not cover the full range of challenges faced when managing a commercial operation.

Overall, the combined improvements highlighted in this report and associated learnings have contributed to improving the average survival out of the hatchery by approximately 220% as identified in Table 6.2. Comprehensive rostering through and improved scheduling during larval stages through 2020 and 2021 was theorised as the single biggest contributor to more successful larval survival, allowing for better observation, timely decision making and overall improved quality of animal husbandry.

On completion of the project many factors, some highlighted in Table 7.1 still need to be addressed. Without further exploration of these factors there is little confidence that Macrobrachium spinipies could achieve production capabilities supporting a financial sustainability.

While the physical outcomes don't represent economical production outputs, they do show some significant improvement in hatchery production and it could be expected that if the range of identified improvements could be addressed and an effective scale achieved, then productive farming of Cherabin could be a reality.

Table 6.2 - Yearly Survival Comparison

	Min	Max	Average
2019	0	1,700	591
2020	0	2,100	585
2021	0	10,000	1,339

Correlating reports from Honey and Fox and other marketing specialist place a premium price on Cherabin products, especially if marketed in a traditional bush foods context which could improve the ability for operators to establish market share in the Australian seafood sector, where a current gap exists as there is no commercial catch for cherabin in the wild sector.

These recommendations should provide any start-up business a sound mechanism to establish pilot scale operations and enable real operational outcomes to be assessed for ongoing feasibility.

7.0 Appendices

Appendix 1.1 - Feed Supplement 1

Customised Egg Custard Supplement

Ingredients:

- Spinach (frozen) x 45g
- Prawns (peeled and thawed) x 105g
- Corn (corn meal , not processed starch) x 180g
- Eggs x 12
- Egg shells x 4
- Enriched artemia x 100g (should be 135g but was 35g short)
- Squid (skinned and gutted) x 105g
- Mussels x 105g
- Liver (chicken) x 105g
- Rice (brown rice was used but preferably white rice) x 150g
- Vitamin premix 1% (a bottle of multi vitamins was purchased from the pharmacy)

Method

1. All ingredients were measured out separately
2. The rice was cooked separately.
3. The egg shells were crushed by hand prior to adding to the mix
4. The following ingredients were mixed together and thoroughly blended in the food processor
    - Spinach
    - Prawns
    - Corn
    - Egg shells
    - Artemia
    - Squid
    - Mussels
    - Liver
5. 150g of cooked rice was added to the eggs and blended separately and then combined with the other ingredients.
6. Multi vitamins were dissolved in 50ml warm water prior to mixing with other ingredients
7. The mixture was again thoroughly blended and then cooked in a microwave at 50%power for around 4 minutes.
8. Once the mixture was cooked it was placed inside plastic bags and rolled to around 10mm thick. This was to get rid of any air bubbles to allow the feed to sink.

Improvements

- The egg shells need to be blended finer, perhaps in a mortar and pestle.
- Further investigate is needed to determine whether the multi vitamin mix is broken down when cooking the diet.

Appendix 1.2 – Feed supplement 2

*As described in Kovalenko E.E et al. / Aquaculture 210 (2002) page 388

Ingredient composition (% dry weight) and results of proximate analysis of the experimental microbound diet

Ingredient	(%)	Source
Egg yolk	38.45	–
Casein (vitamin free)	14.69	A
Fish protein hydrolysate	15.38	B
Rice starch	7.69	C
Refined soy lecithin	1.92	D
Wheat gluten	3.85	C
Menhaden oil	5.63	C
Canthaxanthin (10%)	2.31	E
Cholesterol	0.12	C
Ascorbylpalmitate	0.04	C
Vitamin premix BML #2	1.15	A
Betaine	0.15	C
KH_2PO_4	1.15	C
Choline chloride	0.38	C
Mineral premix	1.54	A
Glucosamine	0.15	C
Alginate	5.38	F
Moisture	62.5	
Protein	46.1[a]	
Lipid	37.4[a]	
Ash	5.6[a]	
Nitrogen free extract (NFE) (by difference)	10.9[a]	

A = ICN Biomedicals (Costa Mesa, CA, USA).
B = Shur-Gain Feed Mills (Truro, Nova Scotia, Canada).
C = Sigma (St. Louis, MO, USA).
D = United States Biochemicals (Cleveland, OH, USA).
E = Hoffmann-La Roche (Nutley, NJ, USA).
F = ISP Alginates (San Diego, CA, USA).
[a] As dry weight.

The methodology used to prepare the microbound diet is patent pending. Fish hydrolysate, casein, rice starch, soy lecithin, wheat gluten, and canthaxanthin were added to a beaker containing distilled water (200 ml/100 g of diet) and solubilized. Menhaden oil, in which both cholesterol and ascorbylpalmitate were solubilized, was then added and mixed. Then, a vitamin premix, betaine (attractant), choline chloride, a mineral premix, monopotassium phosphate, and glucosamine were added and mixed. Yolk was separated from the albumin and the yolk membrane of a chicken egg, and then mixed with the other ingredients.

The mixture of all these ingredients was homogenized in a VirTishear homogenizer (The Virtis, Gardiner, NY) for 3 min at 2000 rpm until a smooth consistency was obtained. Then, alginate was added, followed by additional homogenization for 2 min at 2000 rpm. The resulting homogenate was autoclaved at 554 g/cm3 for 20 min.

The autoclaving procedure was designed to promote physical binding of the dietary ingredients through the combination of temperature and pressure and to eliminate potential anti-nutritional factors that might be present in some ingredients. After autoclaving, the diet assumed a consistency of ‘‘custard’’ and had a moisture content of 62–65%. Crude protein, crude fat (acid hydrolysis method), ash, and moisture were determined according to standard methods (AOAC, 1990). The diet was stored at 5°C and used within 3 weeks after preparation.

Appendix 2.0 – Example of a larval schedule

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 requir
pH - Maintain 7.7 to 8.1.
DO - > 5ppm
Temp - 27-31°C.

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).