Identify factors influencing the variability of survivorship of juvenile redclaw crayfish *Cherax quadricarinatus* (von Martens, 1898) in aquaculture

Thesis submitted by:

Damian Peter Rigg BSc. (Hons)

April 20, 2021

For the Degree of: Doctor of Philosophy

College of Science and Engineering, James Cook University

Supervisors

Principal Supervisor: Associate Professor Clive Jones, Centre for Sustainable Tropical Fisheries and Aquaculture, College of Science & Engineering, James Cook University, Cairns, clive.jones@jcu.edu.au

Co-Supervisor: Professor Jamie Seymour, Australian Institute of Tropical Health and Medicine, College of Public Health, Medical and Veterinary Sciences, James Cook University, Cairns, jamie.seymour@jcu.edu.au

Co-Supervisor: Dr. Robert Courtney, Australian Institute of Tropical Health and Medicine, College of Public Health, Medical and Veterinary Sciences, James Cook University, Cairns, robert.courtney@jcu.edu.au

Acknowledgements

My grateful thanks go to Clive Jones for offering me the opportunity and having faith in my ability to complete this PhD, and Jamie Seymour for recommending me, providing a work space and support within his research group. A massive thank you to Rob Courtney for his awesome dedication to helping with the project, nothing was ever too much, thanks for coaching me along and many late-night theoretical aquaculture discussions! Thank you to Jessica Sleeman, Sally Browning, and all the aquarium volunteers who assisted with animal husbandry for my broodstock, Pauline, Tim, Silvia and Kelsey who assisted in the lab, my Sister Rebecca, brother-in-law Marc, my sister Bernadette and brother-in-law Paul, and my mates Andy and Richard, all of whom know far too much about the rigors of a PhD now. A special thank you also for the support from my great friend Linda. My parents Brian and Cynthia would have taken great joy in this achievement, they are sadly no longer with us but I thank them for all the sacrifices made in my upbringing and education which laid the foundation for this achievement. Finally, my son William; just having you on the planet makes everything okay, even if for now you are half a world away.

Statement of the Contribution of Others

Damian Rigg is the primary author of this Thesis and was extensively involved in all aspects of this work under the supervision of: Associate Professor Clive Jones, Professor Jamie Seymour and Dr. Robert Courtney. Additionally, Robert Courtney was involved in experimental procedure for the Biofloc experiments. Jessica Sleeman and Sally Browning assisted with animal husbandry and photography, numerous aquarium volunteers also assisted with animal husbandry. Simon Irvin of CSIRO Bribie Island Research Centre (BIRC) developed and supplied experimental diets. Andrew Jeffs of the National Institute of Water and Atmospheric Research (New Zealand) determined protein and lipids levels for part 6.4 *Proximate composition of juvenile redclaw, protein and lipid content as indices for subsequent growth and survivorship?* Financial support was supplied by AgriFutures Australia (formerly RIRDC) which provided all necessary equipment and funding for travel and conference attendance, AquaVerde Redclaw Farm and Hatchery supplied experimental animals and broodstock. The Australian Postgraduate Award (APA) scholarship provided financial support for Damian Rigg during this candidature. We thank the Freshwater Crayfish editor and the two anonymous reviewers for their useful comments and suggestions on the four published chapters.

Thesis Dedication

This body of work is dedicated to AgriFutures Australia who provided the funding for this project and continued support of the redclaw aquaculture industry, and also Colin and Ursula Valverde for initiating the project and providing on-going support throughout. I also dedicate this Thesis to my son William Rigg, I believe it is an example of what can be achieved through hard work and dedication, and I hope it serves as inspiration for you so that you always strive to achieve your best regardless of the challenge.

Abstract

The Australian redclaw crayfish, *Cherax quadricarinatus*, has been the subject of aquaculture development for more than three decades, however farm production from Australia and around the world has been low due to a lack of production of juveniles for on-growing to marketable size, and variability in survivorship and growth during early juvenile stages. A nursery phase is proposed between the hatchery and pond grow-out to on-grow redclaw to a more robust size to resolve these barriers. The factors identified for a nursery phase were, diet, water temperature, morphology and allometry, and presumed suitable thermal ranges based on respirometry. Examination of biofloc as an additional food source, redclaw protein and lipids proximate composition as an indicator of condition, and habitat provision and stocking densities to mitigate cannibalism were also conducted.

Diet is a critical factor, but little research has been applied to the initial juvenile stages. An experiment was performed over two weeks which evaluated survival and growth of early instar redclaw using four diet treatments; Frippak (commercially-available post-larval shrimp diet), a compound diet formulated by CSIRO, bloodworms and on-grown *Artemia* sp. Bloodworms and *Artemia* sp. produced significantly higher survival, juveniles fed either *Artemia* sp. or Frippak had significantly higher weight increase. Biomass of redclaw juveniles was significantly highest for *Artemia* sp. treatment. High mortality in the Frippak and CSIRO diet treatments occurred between days six and nine of the experiment, around ecdysis, not wholly attributable to nutritional deficiencies but also feed accessibility. *Artemia* sp. and bloodworms promoted highest survival, *Artemia* sp. and Frippak the highest weight gain. The best combination of survival, weight gain and biomass was the *Artemia* sp. diet.

The second factor examined was the thermal regime, this study quantified the effect of water temperature on the growth and survival of redclaw juveniles for a 22 day nursery phase. Temperature had a statistically significant effect on the survival of juveniles, high temperatures were associated with high mortality, and low temperature treatments were associated with very low mortality. Survival was 98% to 100% for craylings held between 18°C and 22°C, and 0% to 6% for craylings at 25°C to 32°C. Mortalities within treatments 25°C to 30°C, corresponded with the initiation of moulting. Change of mass of crayfish was significantly higher with increasing temperature between 18°C and 22°C. A water temperature of 22°C was found optimal for survival and growth in a nursery phase.

Clear definitions of the successive instars from egg onwards are required for a nursery phase, and also clarification of the characteristics and nomenclature for stages through to an advanced juvenile. Due to this a new naming system was proposed for the first six instars (Egg, L1, L2, J1, J2 and J3), based on gross morphology and allometric relationships. Wet mass and ocular carapace length (OCL) were analysed through linear regression, the size of each instar was defined, and descriptions and photographs of the six instars provided a visual reference for identification. Five of the six instars had a significant relationship between wet weight and OCL, both significantly increased for each successive instar. Lyophilized (dry) weight was not significantly different between stages until after instar J1 where endogenous feeding begins, growth of the first four instars in wet weight and OCL, but not dry weight, suggests an endogenous source of nutrition in addition to the yolk supply.

Following on from the work on allometry, culture thermal ranges need to be explored for all six instars using closed-cell respirometry, to produce a thermophysiological profile to quantify suitable thermal regimes. The temperature

ranges from the data show that each instar has its own presumed suitable thermal range, and that culture temperature may need to alter up or down, dependent upon the instar. Eggs show a range from 22°C - 28°C; L1 from 22°C - 31°C; L2 from 20°C - 26°C; J1 from 20°C - 26°C; J2 from 22°C - 28°C; and J3 from 20°C -26°C. The short-term ranges for eggs through to J3 start at 20°C or 22°C, close to the long-term thermal regimes recommended. A study to examine temperature over a longer period of time from J1 to J3 found an optimal temperature of 22°C, here the optimal temperature range start points are 20°C, 22°C and 20°C respectively. Instar L1 has the broadest presumed suitable thermal range in this study of 9 degrees (22°C to 31°C), which may reflect an ontogenetic change to allow for the maternal female to which it is attached to search for food in microhabitats with broad temperature ranges. This study forms an important link to other studies which explored temperature effects over longer time periods, and can now be used as the basis for an energy budget for the first six instars of redclaw.

Three further studies were initiated as pilot studies to map future directions for a nursery phase. A protocol was developed to produce biofloc cultures, seeking to promote natural production of bacteria, algae and protozoans, for food, shading, processing wastes, and disease mitigation. Biofloc cultures were produced within 14 days, the use of crayfish shortened the time to produce the biofloc, and reduced ammonia down to zero at day 20. The redclaw in the biofloc treatment had 1.45 x larger end mean weight, 3 x larger total weight, 27% higher survival, 78% higher weight gain, and 87% higher biomass than a clearwater treatment. Significant cannibalism made it less clear where nutrition came from, however.

Hatchery produced seed redclaw are sold to farmers at the J1 stage, but their nutritional status and fitness for grow-out are unknown. A measure of condition indicating potential for growth and survival of the crayfish would be an excellent tool.

The lipid and protein composition of redclaw juveniles was measured to assess the utility of these metrics as a proxy for physiological condition. Percentage lipids of dry weight was not a good index for condition of growth or for survival either. The percentage protein of dry weight is also not a good index for condition of growth, but the percentage proportion of protein of dry weight may be a good index for survival.

The third unreplicated trial looked at mitigating cannibalism by providing habitat or structure using two types and two sizes of scaled-down habitat and various stocking densities. The bowtie microhabitat provided less survival than the tube microhabitat, however the mesh of the bowtie microhabitats collected the food, promoting a larger weight gain. The tube microhabitat treatments offered significantly higher survival percentage but lower weight gain. The density treatments of 11-52 redclaw per m^2 had no significant effect on either percentage weight gain or percentage survival. Habitat of one to two orders of magnitude larger is likely required, as the survivorship in this study was low across all treatments (8% large bowties – 37% long tubes).

Protocols for a nursery phase were then developed as follows. **1.** Frozen on-grown *Artemia* sp. as a diet and as a reference diet to test other candidates against. **2.** The temperature for a nursery phase to grow from J1 to J3 is 22°C. **3.** The new designation of instars clarifies the nomenclature, descriptions of sizes and weights aid in identifying instars J2 and J3. Wet weight and occipital carapace length can be determined from each other. **4.** The instars from egg through to J3 show some variability in thermal regime, but all at low temperature ranges. Eggs and J2 have a range from 22°C - 28°C, L1 has a range from 22°C - 31°C, L2, J1 and J3 have a range from 20°C - 26°C. **5.** Nursery Duration should be for three weeks or two moults, temperature- dependent. **6.** Biofloc should be used in tanks. **7.** Indices of condition,

holds potential for a way to look at current condition, a predictor for future condition. **8.** Habitat and Density: a promising way to combat cannibalism and use the three-dimensional space more efficiently.

Table of Contents

Tables

Figures

List of Plates

Chapter 1: A Review of Juvenile Redclaw Crayfish *Cherax quadricarinatus* (Von Martens, 1898) Aquaculture; Global Production Practices and Innovation.

A Version of this Chapter has been published as:

Rigg, D., Seymour, J. E., Courtney, R. L., & Jones, C. M. (2020). A review of juvenile redclaw crayfish *Cherax quadricarinatus* (von Martens, 1898) Aquaculture; global production practices and innovation. *Freshwater Crayfish*, *25*(1), 13-30, 2020ISSN: 2076-4324 (Print), 2076-4332 (Online) https://doi.org/10.5869/fc.2020.v25-1.013.

Statement of the Contribution of Others

Damian Rigg is the primary author of this chapter and was extensively involved in all aspects of this work under the supervision of: Associate Professor Clive Jones, Professor Jamie Seymour and Dr. Robert Courtney. Financial support was supplied by AgriFutures Australia (formerly RIRDC) which provided all necessary equipment and funding for travel and conference attendance. The Australian Postgraduate Award (APA) scholarship provided financial support for Damian Rigg during this candidature. We thank the Freshwater Crayfish editor and the two anonymous reviewers for their useful comments and suggestions.

Chapter 1: A Review of Juvenile Redclaw Crayfish *Cherax quadricarinatus* (Von Martens, 1898) Aquaculture; Global Production Practices and Innovation.

1.1 Abstract

The Australian Redclaw crayfish, *Cherax quadricarinatus*, has been the subject of aquaculture development for more than 3 decades. Farm production from Australia and from around the world for this species has been relatively low, as suitable production technology continues to develop. The production of redclaw juveniles for the purpose of stocking to ponds for on-growing to marketable size, has been a particular constraint and new approaches to breeding and mass production of craylings have provided renewed impetus to industry expansion. This paper reviews the literature concerning redclaw juvenile production and provides a status report of current practices and innovations that may support further expansion of redclaw aquaculture.

1.2 Introduction

The world wild-caught seafood catch has reached and may already have exceeded sustainable levels of production in many fisheries worldwide (Henriksson *et al*. 2012). Feeding a projected world population of 9 billion by 2050 will be a challenge faced in all food production (Msangi *et al*. 2013) and aquaculture will take an increasingly larger role in providing seafood protein. It is estimated that by 2030 aquaculture will provide half of all seafood production, possibly rising to an even greater proportion beyond that date (Msangi *et al.* 2013).

Aquaculture in Australia has also expanded to meet these demands, the major components of the aquaculture industry in Australia consisting of marine and freshwater finfish and crustaceans and a range of marine molluscs (Savage and Hobsbawn 2015). Within the freshwater aquaculture category are three freshwater crayfish groups. These groups are comprised of *Cherax* species and include yabbies (*C. destructor, C. albidus*), marron (*C. cainii, C. tenuimanus*) and redclaw (*C. quadricarinatus*). Of these species, redclaw is the only tropical species, and one that has been widely heralded as an excellent candidate for aquaculture. Production of the juvenile stages of redclaw has been a significant constraint to commercial development. The following provides a review of the literature concerning juvenile redclaw aquaculture to collate existing knowledge and identify knowledge gaps that further research might resolve.

1.3 Redclaw Background

Redclaw is the common name of the freshwater crayfish *Cherax quadricarinatus.* This large and attractive crayfish is a native of westerly flowing rivers in far north-western Queensland and the Northern Territory in Australia, as well as areas of southern Papua New Guinea (Jones 1990b; Jones 1990a; Jones and Ruscoe

2002; Webster *et al*. 2004; Bugnot and Lopez Greco 2009; Ghanawi and Saoud 2012; Saoud *et al*. 2013; Zhu *et al*. 2013; Stumpf *et al.* 2014). Well known to the communities of these remote areas, the species was first introduced to the broader public via its launch in the late 1980's in southeast Queensland as an exciting new 'sunrise' aquaculture species (Jones 1990a) and a potential revenue source for farmers.

Redclaw belongs to the family Parastacidae, which is only found in Australia, New Zealand, New Guinea, Madagascar and parts of South America (Ackefors 2000). The genus *Cherax* includes the Yabby (*C. destructor, C. albidus*) found in more southerly, central and western regions of Australia, and the Marron (*C. cainii, C. tenuimanus*), all of which have been assessed and developed for aquaculture. For redclaw, *C. quadricarinatus*, the specific name refers to the four keel-shaped ridges on the cephalothorax, and the common name is derived from the red coloration of a decalcified patch on the outer margin of the chelicerae of sexually mature males.

Aquaculture of redclaw, yabbies and marron in Australia began around the same time in the mid 1980's. The species have much in common biologically, but their specific aquaculture has developed independently. Yabbies are primarily cultured extensively in farm dams, while marron and redclaw are cultured semi-intensively in purpose-built, managed earthen ponds. Marron require temperate conditions for culture and are relatively slow growing, taking 2 or more years to achieve a minimum marketable size. They are advantaged however, by reaching 500g or more (over several years), making them comparable to marine lobsters in the market place. Redclaw have an advantageous combination of attractive aquaculture characteristics including fast growth, under their preferred tropical conditions, reaching 100 to 200g within 12 months of growth. Redclaw is a robust species that is relatively easy to culture, and its

positive aquaculture credentials have resulted in widespread translocation around the world.

1.4 Redclaw Reproductive Biology

The process by which redclaw reproduce provides an advantage to aquaculture production due to its simplicity compared with other crustaceans (Medley *et al*. 1994), including shrimp, prawns and lobsters. The most significant attribute of all freshwater crayfish including redclaw, is the absence of free-living larval stages to manage in cultivation. After the male redclaw deposits a spermatophore, or sperm package on the sternum of the female, eggs are released and within 24 – 48 hours they are fertilized in a temporary brood chamber on the underside of the female's curled abdomen, in a swirling current created by the beating of the pleopods (Jones 1990b). The fifth pair of pereiopods have sharp tips that are used to break open the sperm packet and the released sperm are then also drawn into the brood chamber, within which the fertilization takes place (Jones 1990b). Eggs then become attached to setae, or fine hairs on the pleopods, on the female's abdomen, and go through 10 developmental stages over the next 31 days before hatching (Garcia-Guerrero *et al.* 2003a).

The developmental stages of redclaw from fertilized egg have been well described by García-Guerrero *et al*. (2003a). They term the first hatched stage as post-embryo 1 or stage 11, at 32-36 days after spawning. At this time, all adult appendages are fully formed and present except for the uropods, and the abdomen is paddle-shaped (García-Guerrero *et al*. 2003a). The cephalothorax is larger than the abdomen due to the presence of a yolk sac, which indicates nil feeding activity, and there is no locomotion as the developing crayfish remains attached to the maternal pleopods (García-Guerrero *et al*. 2003a). Post-embryo 2 (stage 12, days 37-41) has the cephalothorax taking its final shape and nearly all the physical characteristics of the

adult are now evident (García-Guerrero *et al*. 2003a), although there is still no feeding or locomotion as the crayfish remains attached to the female (García-Guerrero *et al*. 2003a). A significant change happens at day 42 when the yolk is depleted and the cephalothorax is of final proportion and shape and exogenous feeding begins. Now capable of independent locomotion, these are the earliest stage juveniles, otherwise referred to as post-larval stage 3 or craylings. These craylings progressively leave their mother for brief forays to seek shelter and food (García-Guerrero *et al*. 2003a), becoming fully independent within a week. After their next moult, they are referred to as juveniles until they mature.

1.5 Beneficial Attributes of Redclaw for Commercial Aquaculture

From the earliest investigations into the suitability of redclaw for commercial aquaculture in the early 1980's, through to the present day, redclaw has shown great potential to become a high value food fish (Jones 1989; Jones 1990b). Redclaw are hardy and benefit from physical, biological and commercial properties which translate to a ready adaptability to farming in sub-tropical and tropical areas worldwide. This potentially broad geographic range, coupled with physical robusticity, straightforward life-cycle and production technology as well as a low protein food requirement, mean that they are economic to produce (FAO 2017). Redclaw also have a substantial return in terms of meat yield, returning a meat to body weight ratio of around 30%, which compares advantageously with other commercially valuable crustaceans (Masser and Rouse 1997). Additionally, the flesh texture and flavour of redclaw compares favourably with those of marine species (Bitomsky 2008), and due to the resemblance to marine lobsters, redclaw are positioned at the premium end of the crustacean market (FAO 2017).

1.6 Biological and Behavioural Attributes

Redclaw exhibit many excellent qualities which translate well to aquaculture such as their hardiness in regard to surviving adverse conditions, low intraspecific aggression and low level of destructive burrowing behaviour (Jones 1990a; Masser and Rouse 1997). The species can tolerate a wide range of temperatures from 16 to 32°C (King 1994; Thompson *et al.* 2004; García-Guerrero *et al*. 2013b), however they grow best between 20 and 34°C (Jones 1990b) and will perish at temperatures below 10° and greater than 36°C (FAO 2017). Capacity to tolerate low dissolved oxygen conditions, as low as 1ppm (Masser and Rouse 1997), is a further advantage. One of the most significant positive attributes applicable to all freshwater crayfish including redclaw, is the lack of free-living larval stages, these being completed within the egg (Jones 1990b; FAO 2017), rather than as independent larvae requiring specific food and environmental conditions (Jones 1995b; Thompson *et al*. 2004; Thompson *et al*. 2006). Another attractive attribute of redclaw is their relatively fast growth rate, reaching marketable size of 60 – 200g within 9 months (Thompson *et al*. 2004; FAO 2017).

Harvesting of aquacultured redclaw is most commonly performed using a flowtrap as described by Jones and Curtis (1994) that comprises a closed box with an attached ramp and a water flow down the ramp. The redclaw walk up the ramp against the water flow and into the 'flow trap' (Jones and Curtis 1994). Redclaw are positively rheotactic with a very strong response to water flow (Jones 1990a). In their natural habitat they inhabit permanent water in the upper reaches of streams and rivers, often in discrete water holes (referred to in Australia by their Indigenous name – billabong) that form during the dry season when rivers are not flowing. Their natural response to walk against the water flow enables them move towards the permanent water if they're

swept downstream during the wet season (FAO 2017). This behaviour has been harnessed for aquaculture, providing an effective and efficient method of harvesting.

1.7 Redclaw Aquaculture in Australia

At present redclaw are farmed commercially on 22 licensed farms in Queensland (Queensland Government 2020) stretching from the Atherton Tablelands in the far north, down to the Sunshine Coast and State border areas in the extreme south of the Queensland. In the financial year 2018 – 19, production decreased by 8.1% from 48.8t in 2017-18 to 44.9t and value decreased from A$1.3M to A$1.2M (Queensland Government 2020). The average price per kilogram also decreased from $26.06 in 2017-18 to $25.69 in 2018-19 (Queensland Government 2020). Over time there have been large fluctuations in production (Figure 1) and the industry has failed to gather new momentum from a high of 105t in 2005 – 06 (Queensland Government 2016).

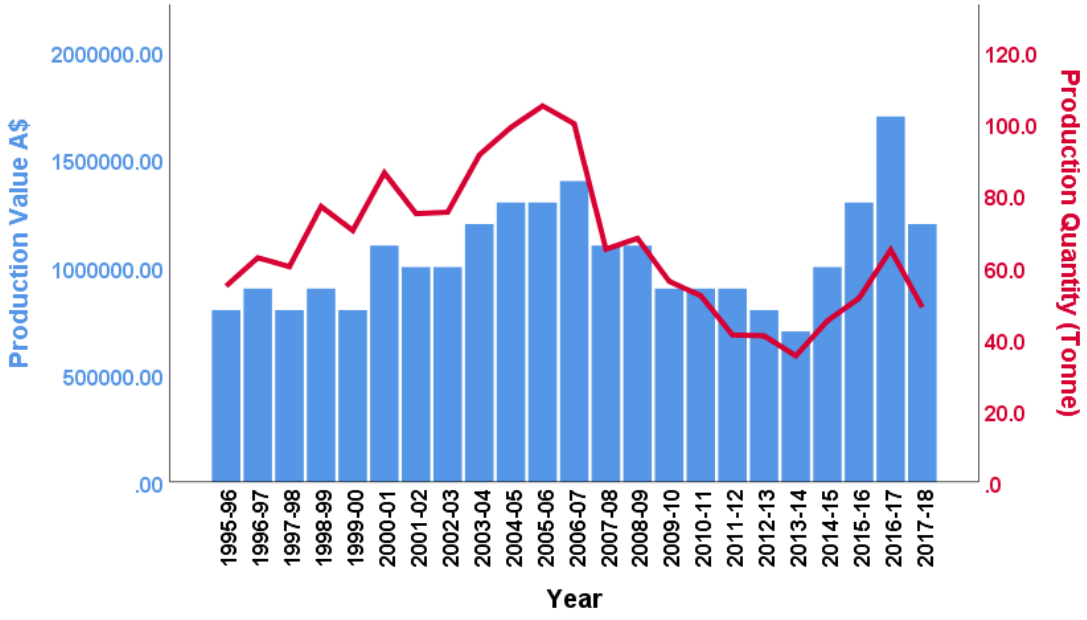

Figure 1. The trend in Queensland redclaw production quantity (tonne) and value (Australian Dollars) from 1995 to 2018 (Queensland Government 2015, 2016, 2018).

The reasons for the fluctuations in both the production volume of redclaw produced in Queensland each year (Figure 1) and the price per tonne (Figure 2) are unclear. After production steadily increased over the period from 1995-96 to 2006-07, there was an equivalent decrease from 2006-07 to 2014-15, followed by an increase to 2016-17 and a subsequent drop again in 2017-18. Despite the production volume variability, the price per tonne (Figure 2) has generally trended upward. This may suggest that the price is decoupled from the production output and that price is not demand-driven. Other factors that could explain this redclaw production variation may be environmental factors such as rainfall, the investment climate with factors such as government subsidies and bank interest rates.

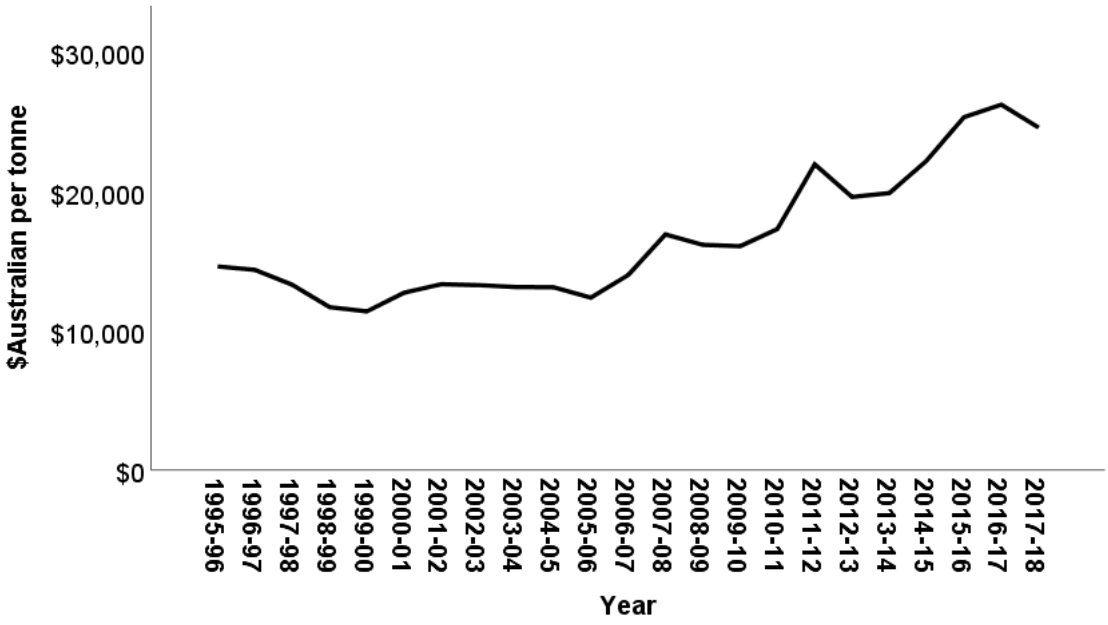

Figure 2. The trend in Queensland redclaw production in Australian Dollars per tonne from 1995 to 2018 (Queensland Government 2015, 2016, 2018).

Redclaw farmers have indicated the availability of seedstock (juvenile redclaw) as a constraint, with most farmers having to produce their own juveniles rather than purchase them from a seedstock supplier – as per most other successful aquaculture industries.

1.8 Other Favourable Aquaculture Attributes

Other favourable attributes of redclaw for commercial production in aquaculture include:

High fecundity, each adult female is able to produce three to five clutches of 300 to 800+ eggs per summer breeding season, and potentially more if environmental breeding conditions are extended (Jones 1995b; Barki *et al*. 1997; Barki and Karplus 1999; Levi *et al*. 1999; Karplus *et al*. 2003; Bugnot and Lopez Greco 2009; FAO 2017).

Disparate wild population strains have provided the basis for selective breeding for improved aquaculture attributes (Jones 1990b; Bitomsky 2008; Stevenson *et al*. 2013; FAO 2017).

A tolerance of high stocking densities, which increases net yield (Jones 1990b; Yeh and Rouse 1994; Barki and Karplus 2000; Jones and Ruscoe 2000; Naranjo-Paramo *et al*. 2004; Webster *et al*. 2004; Rodgers *et al.* 2006; FAO 2017).

A low protein diet requirement during the grow-out stage, allowing for cheaper and more sustainable food sources (Guillaume 1997; Cortés-Jacinto *et al*. 2003; Cortés-Jacinto *et al.* 2004; Cortés-Jacinto *et al.* 2004; Thompson *et al*. 2004; Cortes-Jacinto *et al.* 2005; Thompson *et al.* 2006; Metts *et al*. 2007; Pavasovic *et al*. 2007b; Saoud *et al*. 2008; Zenteno-Savín *et al*. 2008; Cortés-Jacinto *et al*. 2009; Garza De Yta *et al*. 2011; FAO 2017).

Potential for partial or complete fishmeal replacement in formulated diets by plant-based or industry waste protein, which can reduce the price of the feed (Kondos 1990; Loya-Javellana *et al*. 1993; Lawrence and Jones 2002; García-Ulloa *et al*. 2003; Muzinic *et al.* 2004; Thompson *et al*. 2004; Campaña-Torres *et al*. 2005; Thompson *et al*. 2006; Gutiérrez and Rodríguez 2010; Garza De Yta *et al*. 2011; Arredondo-Figueroa *et al*. 2013; FAO 2017).

They can survive extended periods out of the water, up to weeks if the temperature and humidity are optimal, and therefore can be transported without water at all stages from egg to adult, thus reducing transport costs (Jones and Ruscoe 1996; FAO 2017).

Production equipment and associated technology requirement is minimal, allowing for greater ease and less cost in setting up an aquaculture facility (Cortés-Jacinto *et al.* 2003; Thompson *et al*. 2005; Saoud *et al*. 2008; FAO 2017).

Redclaw are tolerant of wide variations in water quality variables including pH, dissolved oxygen, temperature and nutrient loads, allowing savings in labour, equipment and chemicals required to mitigate such variation (Thompson *et al*. 2004; FAO 2017).

Redclaw have osmo-regulatory capacity to tolerate salinity of up to 5ppt indefinitely and up to 15ppt for several days (Anson and Rouse 1994; Jones 1995e). This allows for greater geographic range into areas that may have slightly brackish conditions (FAO 2017), and also means redclaw can be purged and cleaned in salty water, which improves transport survival and enhances the flavour (Jones 1989).

Despite some early promotional efforts by the redclaw aquaculture industry, that generated a positive reception in markets in Australia and overseas, the major constraint to marketing has been lack of supply volume (Queensland Government 2007; Bitomsky 2008). Further active marketing is required to increase acceptance and awareness domestically, however current production volumes are still too small to support an export market (Bitomsky 2008).

The potential for aquaculture of redclaw based on climatic conditions extends across the north of Australia from northern Queensland, across the Northern Territory and to the Kimberley area of Western Australia (Queensland Government 2007).

Despite the favourable conditions, the locations where farming has been successful have been confined to northern and southern Queensland, due to their proximity to markets, labour and infrastructure. The areas where farming of redclaw is environmentally favourable in Northern Territory and Western Australia are logistically unsuitable at present due to their remoteness, limited access to labour, markets and infrastructure.

1.9 Global Redclaw Aquaculture

Redclaw has been introduced as an aquaculture species to Argentina, Barbados, Ecuador, Guatemala, Malaysia, Mauritius, Mexico, New Caledonia, Samoa, Swaziland and Uruguay (FAO 2016), to Belize, Indonesia, Morocco, Panama, and Spain (FAO 2017), and to the USA (Masser and Rouse 1993; Ackefors 2000), where it has shown potential for cultivation in the south-eastern states (Rouse and Yeh 1995; Ackefors 2000). There has also been some redclaw aquaculture development in Israel (Karplus *et al.* 1995; Ackefors 2000) and China (Ackefors 1994; Ackefors 2000) but there are no reliable statistics available of production. Anecdotal information (e.g. availability in wet markets) suggests there is a substantial redclaw aquaculture industry in China.

Worldwide reported production of redclaw (excluding possible production from the People's Republic of China and Hong Kong) decreased from 150 tonnes in 2005 to 129 tonnes in 2014 (FAO 2016). There have been various fluctuations in production over the years (Figure 3) and several countries have gone in and out of production (FAO 2016). A good example is Ecuador, where redclaw was introduced for aquaculture with a substantial start-up of 250 ha of ponds in 1994 but had virtually disappeared as an industry by 1998 (Romero 1998; Romero and Jimenez 2002). In Ecuador redclaw production was initially very encouraging (Rouse 1994; Salame 1995; Romero 2002),

but this was confounded by a lower farm-gate price than expected and difficulties with developing a market (Romero 1998; Romero and Jimenez 2002).

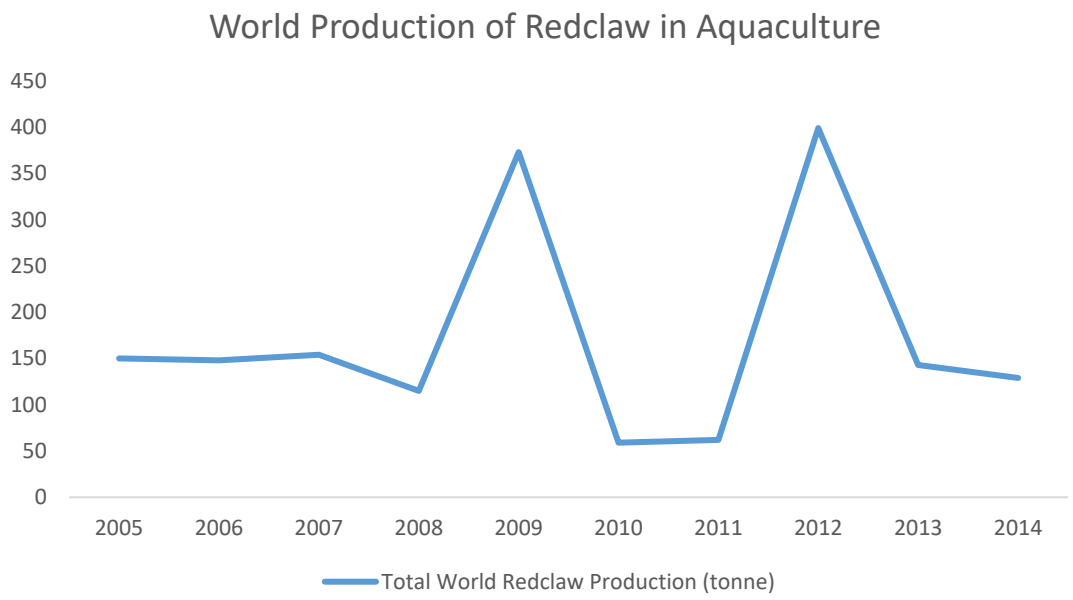

Figure 3. Global redclaw production trends from 2005 to 2014 (FAO 2017).

In many of the countries to which redclaw was translocated, it has become established as a minor aquaculture industry but with little growth (FAO 2017). For example, Mexico produces around 50 tonnes per annum, and the USA, Belize and Panama which each produce less than 10 tonnes per annum (FAO 2016). Ecuador had significant production of juveniles briefly in the late 1990's for stocking new farms, but output is now negligible. Substantial infrastructure for large scale redclaw farming activities was constructed in Spain and Morocco in the late 1990's and early 200's, but the subsequent production is unknown (FAO 2017) and likely to be insignificant.

Due to the positive characteristics which make redclaw a favourable aquaculture species and the broad tolerance of environmental conditions, they have been translocated to 67 countries or territories and have established wild populations in 22

countries, having been reported on every continent except Antarctica (Haubrock *et al.* 2021). This introduces a note of caution for aquaculture, in that there is potential for wild populations to establish in their non-native range, as well as the potential to introduce parasites such as non-native temnocephalids, or to become a vector for spread of the crayfish plague *Aphanomyces astaci* (Hsieh *et al.* 2016).

1.10 Global Cultivation of Redclaw Seedstock

Outside of Australia redclaw aquaculture is limited, and information on production of juvenile redclaw for supply to grow-out operations is scant. Various studies have investigated the biology and the feasibility of redclaw aquaculture in other countries, including those of Yeh and Rouse (1994); Abdu *et al*. (1997) and Rodríguez-Canto *et al.* (2002). However, few studies have examined the production of juveniles (Parnes and Sagi 2002). Calvo *et al*. (2011) described a method of producing craylings and one gram juveniles for use in an experiment in Argentina. In this study berried females carrying attached eggs (ovigerous), were placed individually in aquaria and monitored for development. Once the juveniles had reached the free-living, post larval stage 3 (crayling) they were separated from the female and transferred into experimental apparatus (Calvo *et al*. 2011). In another study, juvenile production was examined, but in a semi-intensive way and concentrated on assessing the optimum density and juvenile habitat requirements (Parnes and Sagi 2002).

In Australia there are a range of methods of cultivation of juvenile redclaw from extensive to intensive (Jones 1995b, c) and in the absence of strong evidence otherwise it is assumed that this is also the case in overseas production (O'Sullivan *et al.* 2003). The intensification of juvenile production may be of benefit to developing markets outside of Australia as well as within, incorporating a 'best-practice' approach based

on rigorous science to enhance farming efficiencies, profit and eventual expansion of the redclaw industry.

1.11 Early Juvenile Redclaw Production, the late 1980'S

The initial extensive method of producing redclaw juveniles involved little intervention in the natural breeding process, relying instead on the natural, seasonal reproduction under ambient conditions. Farmers stocked production ponds with a ratio of 4 adult females to 1 adult male, and simply allowed them to breed during summer (Stevenson *et al*. 2013) and produce offspring naturally to stock the pond. Development of separate juvenile cultivation techniques was considered unnecessary (Jones 1995b).

A confounding outcome of this extensive and straightforward approach to producing juveniles for grow-out was repetitive and asynchronous breeding that generated multiple cohorts (Sammy 1988; Karplus *et al*. 2003) within the pond. Harvesting of the production ponds revealed the multiple cohorts and significant size variation of crayfish, which is not conducive to generating a crop of consistent marketable size redclaw.

This approach, allowing natural breeding in the grow-out ponds, generates the additional juveniles and increased and uncontrolled density of stock, which likely results in density-dependent growth inhibition (Barki *et al*. 2006) and resource competition between cohorts (Jones and Ruscoe 2000). Furthermore, the disparate sizes of the crayfish and unsynchronised molting likely contributes to increased cannibalism, as small, soft post-moult crayfish are particularly vulnerable to predation by conspecifics. Therefore, in spite of the multiple breeding events, juvenile survival was low, estimated by Jones (1995b) to be no more than 5 – 10%.

A further problem with extensive methods of producing juveniles was potential for inbreeding due to the selection of the broodstock. Broodstock selected as the larger

and fitter specimens from previous harvests are likely to be closely related (McPhee *et al*. 2004). Adult broodstock left in broodstock ponds throughout a summer grow-out period for juvenile production represents an opportunity cost, as these animals are unable to be sold (Stevenson *et al*. 2013). Using the extensive methods, output of juveniles is inconsistent as a function of the number and quality of berried females, the influence of seasonal factors (day length, water temperature), and variability in stocking (Stevenson *et al*. 2013).

1.12 The Next Phase in Juvenile Redclaw Production, the late 1990's

A more controlled procedure was subsequently adopted whereby male and female brood stock, selected for size, growth and vigour, were introduced into specifically designated broodstock ponds at a ratio of 1 adult male to 4 adult females (Stevenson *et al*. 2013) and density of 1 adult per square metre. Once it was established that the females were berried they were separated from the males and stocked to grow-out ponds (Stevenson *et al.* 2013), where the offspring would be released naturally, to stock the pond. A similar method was used by Jones (1995c) where berried females were selected and staged according to their egg colour (Jones 1990b; Jones 1995b). Females with eggs at a similar stage were stocked into a juvenile production pond. This method represented an advance in intensification that enabled a single cohort to be generated and avoiding further breeding due to absence of any mature males.

Another variation on this method involved sourcing the berried females from the harvest of other grow-out ponds (Stevenson *et al*. 2013). These berried females were stocked to ponds and left for 6 – 12 weeks to enable the eggs to hatch and juveniles to develop to an advanced stage of 5 – 15 grams, whereupon they were harvested, graded by size and then used to stock grow-out ponds (Stevenson *et al.* 2013). A major problem

with this approach was the difficulty of estimating the age of an animal from its size. Some of the 'juveniles' were likely to be runts - slow-growing adults (Stevenson *et al*. 2013), that would continue to grow slowly and therefore lower the value of the crop at harvest.

A variety of methods has also been applied to juvenile production for research purposes. Jones and Ruscoe (2000) produced advanced juveniles in ponds stocked with mature broodstock and left for 4 months, flow-trapping them (Jones and Curtis 1994) for grow-out experiments to examine density and size at stocking effects in earthen pond conditions. McPhee *et al*. (2004) produced craylings for experiments by inducing redclaw to mate and become berried in fiberglass breeding tanks. Water temperature (26 – 28°C) and day length (14 hours light, 10 hours dark) were maintained to simulate mid-summer breeding conditions (McPhee *et al. 2*004), and berried females were then transferred to pens constructed of plastic mesh and shade cloth within traditional earthen grow-out ponds. After 4 months, advanced juveniles and female broodstock were harvested and separated (McPhee *et al.* 2004). At this point in the evolution of juvenile production techniques for redclaw for use in aquaculture in Australia, it appeared necessary to take more control of the production to achieve more predictability of output, to minimise disease transmission, to improve genetic quality (Stevenson *et al.* 2013) and to enable all farm ponds to be allocated for grow-out rather than having a proportion occupied for juvenile production.

1.13 Advancement in Methods of Juvenile Redclaw Production

In 2005 the redclaw industry in north Queensland (Australia) started to embrace further intensification of juvenile production, including control of egg incubation. To

facilitate this, North Queensland Crayfish Farmer's Association (NQCFA) member AquaVerde imported the Hemputin incubator system from Finland (Jones and Valverde, 2020; Stevenson *et al*. 2013). Managed incubation was achieved by removing the eggs from the pleopods of the female at the mid-stage around 4 weeks after fertilization, then incubating them in the Hemputin system, specifically modified for redclaw. The eggs from each female (around 300 – 800 eggs) were held in small (100ml) plastic baskets, placed in rack in a shallow water bath with uni-directional water flow, connected to a recirculation system with UV sterilization and biological filter. Once the eggs hatch, they are left in the incubator baskets for 2 moults, until the crayling stage is reached. The craylings thus produced are robust and suitable for transport. The advantages of this artificial incubation approach, were that the eggs could be treated to reduce pathogens, processed in discrete cohorts and batched for commercial orders. This then allowed redclaw grow-out farmers to purchase such hatchery generated craylings and allocate all of their ponds to on-growing, ordering craylings for new stockings as required.

A significant improvement in management that hatchery-produced craylings provided was that batches of a fixed number, of known-age craylings could be introduced to the grow-out ponds to achieve a specific density of stock in the pond. By harvesting such ponds at 6 to 9 months (age at first maturity), there is little likelihood of any breeding that would confound the stocking (Barki *et al*. 2006). Because the hatchery reared craylings would be of same age and size, the consistency would help mitigate cannibalism, as the crayfish would moult more synchronously (Ghanawi and Saoud 2012). This in stark contrast with earlier on-farm juvenile production, which typically involved stocking with wide variation in size and therefore increased opportunity for cannibalism (Ghanawi and Saoud 2012). A further advantage of the

hatchery approach, was the capacity to select crayfish for breeding that demonstrated superior characteristics, including size at age and robustness, leading to stock improvement over time.

With the establishment of a hatchery supply of craylings, redclaw farmers now have the opportunity to focus entirely on grow-out, purchasing seedstock only as required. This is a major advancement that brings redclaw aquaculture in line with other successful aquaculture industries that have dedicated hatcheries to supply the seedstock (Jones and Valverde, 2020; Stevenson *et al*. 2013).

Predictable and consistent seedstock production from hatchery operations is likely a key factor in the expansion of redclaw aquaculture both within Australia, and abroad. Nevertheless, the hatchery technology is relatively new and the protocols and methods require further refinement. It remains unclear whether stocking craylings to grow-out ponds is optimal or if an intermediary nursing phase is necessary to on-grow craylings to advanced juvenile stage prior to pond stocking. There is justifiable concern that craylings are highly vulnerable to predation, and that survival through the grow-out may be improved by stocking grow-out ponds at a more advanced juvenile stage. Research will be necessary to resolve this issue, learning from previous research on redclaw seedstock production methods and performing new research to define a best-practice model for producing juveniles for grow-out operations. The aspects requiring further research to perfect the hatchery approach for redclaw include; nursery diet, optimal nursery period, temperature, provision of habitat, stocking density and prevention of cannibalism.

1.14 Nursery Diet

A considerable body of research has been published on redclaw nutrition for the grow-out phase, from juvenile (Jones 1995a, d, c, b; Meade and Watts 1995; Anson and

Rouse 1996; Fletcher and Warburton 1997; Loya-Javellana and Fielder 1997; Ruscoe *et al*. 2000; Cortés-Jacinto *et al.* 2003; Thompson *et al*. 2003b; Thompson *et al.* 2003a; Cortés-Jacinto *et al*. 2004; Hernandez *et al.* 2004; Muzinic *et al*. 2004; Thompson *et al*. 2004; Cortes-Jacinto *et al*. 2005; Lopez-Lopez *et al*. 2005; Thompson *et al.* 2005; Campana-Torres *et al.* 2006; Metts *et al*. 2007; Saoud *et al*. 2008; Zenteno-Savín *et al*. 2008; Gutiérrez and Rodríguez 2010; Thompson *et al.* 2010; Garza De Yta *et al*. 2011; Saoud *et al*. 2012; Viau *et al.* 2012; Zhu *et al*. 2013; Dammannagoda *et al.* 2015; Pirozzi *et al*. 2015; Volpe *et al*. 2015) through to marketable size (*c.*100g) (Loya-Javellana *et al.* 1993; Asgari 2004; Pavasovic *et al.* 2006; Pavasovic *et al.* 2007a, b; Campaña-Torres *et al*. 2008; Rodriguez-Gonzalez *et al.* 2009a; Rodriguez-Gonzalez *et al.* 2009b; Li *et al*. 2011; Saoud *et al*. 2012; Pirozzi *et al*. 2015). As food can constitute up to 70% of the operating cost in aquaculture (Thompson *et al*. 2003a; Metts *et al*. 2007) cost-effectiveness of the feed is a critical factor for aquaculture worldwide. The rising cost and falling supply of fishmeal (and issues regarding its source and sustainability) have stimulated research experiments with redclaw to find cheaper and sustainable alternatives. Studies have now examined plant-based and terrestrial animal sources for the protein component in aqua feeds (Jones and Ruscoe 1996; García-Ulloa *et al.* 2003; Muzinic *et al*. 2004; Campaña-Torres *et al*. 2005; Thompson *et al*. 2005; Thompson *et al.* 2006; Metts *et al.* 2007; Gutiérrez and Rodríguez 2010; Ranjan and Bavitha 2015). A number of studies have demonstrated that not only can the protein be of plant origin, but the palatability and efficacy of such diets for the grow-out of redclaw is high (Muzinic *et al*. 2004; Thompson *et al.* 2005; Thompson *et al*. 2006).

The protein requirement for good growth and efficient food conversion (food conversion ration FCR = Feed Given / Animal Weight Gain) in redclaw has received considerable attention, with a number of studies identifying optimal protein inclusion

rates in the diet (Cortés-Jacinto *et al.* 2003; Cortés-Jacinto *et al.* 2004; Cortés-Jacinto *et al*. 2004; Muzinic *et al*. 2004; Thompson *et al*. 2004; Campaña-Torres *et al*. 2005; Cortes-Jacinto *et al.* 2005; Thompson *et al*. 2005; Rodríguez-González *et al*. 2006; Thompson *et al*. 2006; Metts *et al*. 2007; Pavasovic *et al*. 2007b; Saoud *et al.* 2008; Zenteno-Savín *et al*. 2008; Cortés-Jacinto *et al*. 2009; Rodriguez-Gonzalez *et al*. 2009; Gutiérrez and Rodríguez 2010; Garza De Yta *et al*. 2011; Rodríguez-González *et al*. 2011; Arredondo-Figueroa *et al*. 2013; Stumpf *et al*. 2014; Ranjan and Bavitha 2015) that are specific to the life history stage of the crayfish (Cortés-Jacinto *et al*. 2009). These studies indicate that the protein requirement falls as redclaw ages. Cortés-Jacinto *et al*. (2009) indicate that juvenile redclaw require 31 to 34% protein and pre-adults (<50g) require 25.6% protein (Cortés-Jacinto *et al*. 2004). Jones (1995c) observation that craylings and early stage juveniles <0.6g consume zooplankton, may reflect a higher protein requirement.

Lipids are also a critical component of the diet that affects growth and health, and many studies have examined lipid requirements for redclaw (Hernandez-Vergara *et al*. 2003; Thompson *et al*. 2003b; Thompson *et al*. 2003a; Cortes-Jacinto *et al*. 2005; Campana-Torres *et al*. 2006; Campaña-Torres *et al*. 2008; Zenteno-Savín *et al*. 2008; Rodriguez-Gonzalez *et al*. 2009; Thompson *et al*. 2010; Li *et al*. 2011; Zhu *et al*. 2013). Cortés-Jacinto *et al.* (2003) suggest that the optimal level of dietary digestible lipid is 75g kg^{-1} for small (1.08 +/- 0.34g) juveniles, while others suggest a dietary lipid level of 87g kg^{-1} to optimize egg quality for spawning females (23 +/- 3g) (Rodriguez-Gonzalez *et al*. 2009). Further studies have also examined dietary lipid levels and concluded that 80g kg^{-1} satisfied the requirements for optimal growth, prevented oxidative stress and protected immune function integrity in small juveniles ranging from 0.7 – 1.54g (Cortes-Jacinto *et al*. 2005; Zenteno-Savín *et al*. 2008). Hernandez-

Vergara *et al.* (2003) found that 42g kg^{-1} dietary lipids were acceptable for larger juveniles (4.08 +/- 0.2g) if natural food sources were available to supplement the diet provided. In contrast to protein, the dietary lipid requirement does not appear to vary with the life history stage of redclaw (Guillaume 1997; Campaña 2001; Joaquí and Montes 2001; Cortés-Jacinto *et al.* 2004; Cortes-Jacinto *et al*. 2005; Arredondo-Figueroa *et al*. 2013).

Carbohydrates are another essential component of the diet used to satisfy energy requirements (Zhu *et al*. 2013), and that contribute to the formation of steroids and fatty acids, and assist in glycogen storage and chitin synthesis (Parvathy 1971; Dall *et al.* 1991; Ahamed Ali 1993; Sánchez-Paz *et al*. 2006; Saoud *et al.* 2012). Commercially, there is an economic imperative to maximize carbohydrate inclusion in formulated diets, as it can be used as an inexpensive filler (Saoud *et al*. 2012). Typically, carbohydrate content is maximized and protein and lipid inclusion is minimized on the basis of ingredient cost (Campana-Torres *et al.* 2006; Campaña-Torres *et al*. 2008; Zhu *et al*. 2013) balanced against effective and efficient utilisation for somatic growth (Sedgwick 1979; Campaña-Torres *et al.* 2008). Carbohydrates in the diet can also have a protein sparing effect, preventing catabolism (Sedgwick 1979; D'abramo and Robinson 1989; Pillay 1990; Guillaume and Choubert 2001; Wouters *et al.* 2001; Saoud *et al.* 2012).

Zhu *et al.* (2013) found that the best ratio of carbohydrates to lipids for redclaw was 3.6:1, translating to a proportion of 290.10g kg^{-1} carbohydrates and 80.70g kg^{-1} lipids for optimal digestive and hepatic enzyme activities, body composition and growth performance in juveniles (1.54 +/- 0.02g). Conversely, in digestibility trials, Campana-Torres *et al*. (2006) and Campaña-Torres *et al.* (2008) found that an equivalent proportion of plant-derived carbohydrates (150g kg^{-1} for 3.62 +/- 1.35g

juveniles, 2006 study; 145g kg^{-1} 10 +/- 0.8g pre-adult, 2008 study) containing a high cellulose content were similarly effective for the redclaw of different size. It has been demonstrated that redclaw can assimilate cellulose (Xue *et al.* 1999; Pavasovic *et al.* 2006; Campaña-Torres *et al*. 2008) due to α-amylase cellulose laminarinase activity in the redclaw alimentary tract (Figueiredo *et al*. 2001; Campaña-Torres *et al*. 2008) and the presence of *p*-nitrophenyl glycosidases in the gastric fluids (Figueiredo *et al*. 2001; Campaña-Torres *et al*. 2008).

Despite the considerable body of work on examining carbohydrate requirements for redclaw, none of it has applied to craylings (≤0.02g). Thompson *et al.* (2003a) conducted an experiment with craylings at 0.02g but examined only practical diets with or without supplemental lecithin or cholesterol. Other studies assessing carbohydrate requirement either linked them to lipids (Campaña-Torres *et al*. 2008; Zhu *et al*. 2013) and / or used larger juveniles (Campana-Torres *et al*. 2006; Zhu *et al*. 2013) or sub adult animals (Campaña-Torres *et al*. 2008). There is a knowledge gap concerning the appropriate proportion of carbohydrate in the diet of craylings, that is likely to be of importance due to the known ontogenetic diet shift which most crayfish exhibit (Saoud *et al*. 2012). Adult freshwater crayfish generally consume greater amounts of macrophytes and detritus in their diets, whereas juveniles feed largely on invertebrates (Mason 1975; Loya-Javellana *et al*. 1993; Lodge and Hill 1994; Momot 1995; Nystrom 2002; Saoud *et al*. 2012).

Figueiredo and Anderson (2003) reported that small juvenile redclaw from 5mm (carapace length) which had been recently released from the female had high levels of protease and low levels of carbohydrases in their hepatopancreas, which reversed in abundance in larger animals, presumably as there was increased preference for plant-derived food as the crayfish grew. Carbohydrase activity increased in redclaw up to

100mm in length peaking at 140mm (total length) (Figueiredo and Anderson 2003) which is the size where a preference for plant material has been documented (Figueiredo and Anderson 2003). Cellulase was present in all free-living stages indicating an ability to digest cellulose at all life stages (Figueiredo and Anderson 2003). The correlations between enzyme levels and diet and feeding habits reflect the development stage of the animal and the morphological changes which are occurring in the gut (Lovett and Felder 1990; Figueiredo and Anderson 2003), and hence the ontogenetic diet shift (Saoud *et al.* 2012). The evidence is clear for an ontogenetic shift in the dietary requirements from juveniles to adult, but little is known about the specific requirements of the crayling stage.

1.15 Ontogenetic Diet Change

There are challenges in developing manufactured diets that account for the specific nutrition required at successive life stages following the transition from endogenous to exogenous feeding (García-Guerrero *et al.* 2003) and through the ontogenetic and developmental changes (Lovett and Felder 1990; Figueiredo and Anderson 2003) from crayling to advanced juvenile and on to adult. It is likely that different diet formulations will be required for different life stages of redclaw.

There are several physical properties for a manufactured diet which also warrant discussion. A nursery diet which takes redclaw craylings through to a more advanced size would require good stability and durability in water due to the periodic nature of feeding (Ruscoe *et al*. 2005) and texture, pellet size and moisture content also require evaluation for suitability (Ruscoe *et al*. 2005; Volpe *et al*. 2015). Furthermore, the morphology and mechanical abilities of the mouthparts of juveniles and large adults have been explored (Loya-Javellana and Fielder 1997) and this information needs to be applied to the manufactured diet.

Early juvenile redclaw have sharply pointed teeth on the small, third maxilliped and mandible, useful in handling small animals as food, combined with reasonably long setae around the margins of the mouthparts which could be used to capture prey (Barker and Gibson 1977; Lavalli and Factor 1992, 1995; Loya-Javellana and Fielder 1997). The anterior pointed accessory tooth to the left of the incisor ridge in young juveniles may also be consistent with raptorial feeding (Loya-Javellana and Fielder 1997). As redclaw develop into young adults the teeth become less pointed but larger, reflecting a decline in small animals as food, increasing macrophagy and an ability to cut plant material (Loya-Javellana and Fielder 1997). The capabilities of the mouthparts at these particular stages should be taken into account in the design of the manufactured food so it best meets their morphological capacity.

Another approach which has been examined for crayling and early juvenile diet is to look at natural food sources, using them exclusively or supplemented with a manufactured feed. Anson and Rouse (1996) trialled and compared various commercially-produced feeds (crawfish-feed, rabbit-chow, trout-chow, shrimp-feed, Biodiet, crustacean reference diet, Shrimp-el-etts) with live *Artemia* nauplii, catfish muscle and squid mantle, and found that for the first two weeks, craylings and early stage juveniles (*c.*20mg) showed improved growth and survival when fed *Artemia* or a combination of *Artemia* and a commercial diet. Jones (1995c) performed a 39 day trial on newly-hatched (*c.*20mg) craylings comparing fresh zooplankton (comprising cladocerans [*Moina* spp.], copepods, chironomid larvae) with commercially produced 'Frippak' Flake and found that zooplankton supported the best growth. A confounding result however, was poor survival, which was unaccounted for but appeared to be related to an interaction with a floating water plant *Pistia stratiodes,* that was provided as a shelter. Meade and Watts (1995) compared commercial, formulated feeds (AB

UAB Research Foundation Formulation, Crayfish Feed, Catfish Floater, Shrimp Grower, Post-larval Granules and Shrimp Grower Pellets) with naturally-sourced feeds (brine shrimp flakes, freeze-dried krill, hatchfry encapsulation, powdered spirulina) and found the best results over 10 weeks with the AB feed (30% protein, 10% lipids, 10% carbohydrate). Substantial weight gain was recorded, combined with a survival of >95% and it supported the notion that the culture of redclaw juveniles can be successful using formulated feeds (Meade and Watts 1995). It should be noted that the 10 week trial period of the Meade and Watts (1995) research, may have skewed the results as any advantage of the 'natural' feeds may have been significant only in the first two weeks as per Anson and Rouse (1996). They suggested the 'naturally-sourced' feeds offerred may have been undigestible during later developmental stages (Campana-Torres *et al*. 2006) or had become unpalatable.

Although there appears to be evidence that natural feeds, especially zooplankton (Tcherkashina 1977; Jones 1995c) and decayed plant material (which may hold epibenthic and sessile epiphytic organisms), may have a benefit in crayling and early juvenile growth and survival (D'abramo *et al*. 1985; Celada *et al*. 1989; Mitchell and Collins 1989; Brown *et al.* 1992; Mcclain *et al.* 1992; Loya-Javellana *et al*. 1993), supplemental feeding with a nutritionally-balanced manufactured feed as the juveniles grow older is likely to provide essential nutrients for maximum growth and be better assimilated as they age (Anson and Rouse 1996). It would be useful to gather data on feeding craylings and early stage juveniles not only different diet formulations, but also incorporating the food ration and feeding frequency to determine optimal feeding practice.

For the advancement of the redclaw industry, further research is required, specifically targeting the crayling and early juvenile stages, on development of an

effective diet formulation, natural feed or combination diet, source of ingredients, the inclusion rates of protein, lipids and carbohydrates, and the feeding husbandry in regard to rations and feeding frequency.

1.16 Nursery Phase

Commercial aquaculture industries for most crustaceans involve distinct phases from hatchery through nursery to grow-out (Parnes and Sagi 2002). The development of commercial redclaw hatcheries in Australia revealed challenges with improving the vigour and resiliance of craylings destined for release into grow-out ponds (Stevenson *et al*. 2013). Recently-hatched craylings stocked directly into grow-out ponds can result in poor and unpredictable survival rates (Garza De Yta 2009). Holding craylings for a nursery period prior to release into grow-out ponds may hold benefits in terms of subsequent survival and growth to harvest (Garza De Yta 2009). It is envisaged the nursery phase would nurture the crayling to an advanced juvenile size, sufficiently robust for on-growing. Redclaw growers in Mexico typically stock grow-out ponds with juveniles above 1g to ensure higher survival rates (Garza De Yta 2009), for the same reasons an early study in Australia (Jones and Ruscoe 1996) recommended stocking advanced juveniles between 5 and 10g. The nursery phase for many species is typically high cost, due to high density and intensive management, therefore its duration should be limited. Jones (1995b) examined holding newly released craylings for a nursery period in individual tanks with two types of habitat: fiberglass fly-screen mesh strips suspended from floats; and timber frames with fly-screen mesh strips woven through plastic trellis mesh (Smith and Sandifer 1979; Jones 1995b). The feeding regime was commercial Flake ‘Frippak’ combined with proportions of either fresh or frozen zooplankton. The highest mean size of 0.427g was achieved in 41 days at a survival rate of 52.3% fed ‘Frippak’ plus 100% frozen zooplankton (Jones 1995b).

Parnes and Sagi (2002) examined a nursery period for newly-released craylings from closely synchronised ovigerous female broodstock, where they utilised seaweed-like plastic elements for habitat within fibreglass tanks. Over a period of 35-40 days the craylings were fed grated potatoes, carrots and commercial fish pellets; the average male and female weights at the end were 0.54 ± 0.02g (n = 432) and 0.49 ± 0.02g (n = 376) (Parnes and Sagi 2002). This study showed that by the addition of a three dimensional habitat / substrate, craylings would effectively utilise almost the entire volume of the tank, thereby increasing the stocking capacity as compared to the two dimensional benthic space of a tank with no habitat / substrate, and that a uniform-sized crayling population would minimise cannibalistic interactions between conspecifics.

In a series of three experiments in Alabama, USA, Garza De Yta (2009) evaluated different hatchery-nursery proceedures to test for maximum survival, final weight and production of advanced juveniles. The factors were water depth, broodstock stocking density and nursery period duration. In the first experiment tanks were stocked with nine females per tank at a 100mm or 200mm water depth or 18 females at 200mm. A 31 day nursery period was initiated after all the craylings had released from the female broodstock within a 96 hour time period. Craylings were fed commercial crayfish pellets (30% protein, 8% lipid) at a rate of 10% of body weight and provided with bundles of onion-bag mesh for shelter. The second experiment held 8, 12, 16, 20 or 24 berried females (densities of 2.8, 4.2, 5.6, 6.9 and 8.4 females per m^2) in similar tanks with the same protocols and a 30 day nursery period was applied. In the same way a third experiment tested stocking 8, 12, 16 berried females (2.8, 4.2, 5.6 females per m^2) for nursery periods of 20, 30 or 40 days.

At the conclusion of this set of experiments it was found that a stocking density of 12, 16 or 20 berried females per tank (4.2, 5.6 or 6.9 females per m^2) produced the

best output in terms of juvenile production based on survival and average weight in a hatchery / nursery period (Garza De Yta 2009). Survival was best at 30 days and as the growth appeared to slow from 30 to 40 days and survival decreased at 40 days, the optimal period for the hatchery / nursery phase was 30 days (Garza De Yta 2009). Water depth as a treatment only indicated no significant effect. Unfortunately, habitat / shelter provision for the juveniles in the form of onion bag mesh was not used as a treatment, and this may have had an effect on the survival and harvest weight of the juveniles, and a potentially confounding effect on the results. If onion bag mesh had been provided for in terms of expected number of juveniles, it may have clarified the results.

Although the Garza De Yta (2009) survival rates were lower compared to other nursery experiments (Jones 1995b; Masser and Rouse 1997) the size of the craylings at 30 days was comparable to the size of craylings produced by Jones (1995b) and Parnes and Sagi (2002) over a period of 41 and 40 days respectively (Jones 0.427 g, Parnes and Sagi [male] 0.54 ± 0.02 g [female] 0.49 ± 0.02 g). Regardless of the effect of female broodstock density the mean total production, survival and therefore final biomass was best at the 30 days (Garza De Yta 2009). This nursery duration maximised the total production of advanced craylings without sacrificing average weight output (Garza De Yta 2009). However, the harvest size and survival rates for hatchery-nursery phase require substantial increases (Garza De Yta 2009) to assist the advancement and development of the production of redclaw seedstock.

1.17 Temperature

Environmental temperature is an integral component of the physiological capacity of an organism to consume and convert resources such as food into growth, reproduction and survival. This is particularly important for organisms which cannot control their internal body temperature, and are forced to match their environmental

temperature. This is the case for thermoconformers in marine and freshwater environments which are beholden to environmental thermal regimes.

Redclaw crayfish are thermoconformers and like other arthropods such as insects, development and growth are positively correlated with temperature, within an optimum range. For insects the concept of "degree days" was developed (Higley *et al*. 1986) to describe the rate of development over time, in relation to temperature. For redclaw, the duration of the successive developmental stages from egg hatch, through two moults to crayling, and through each juvenile stage will be correlated with temperature. For development of nursery technology, it is essential to identify the most advantageous temperature for each stage, balancing development time and survival (Jones 1990b; King 1994; Yeh and Rouse 1994; Jones 1995c, b; Zhao *et al*. 2000; Garcia-Guerrero *et al*. 2003a; Karplus *et al*. 2003; De Bock and López Greco 2009).

Vazquez *et al*. (2004) showed that it is possible to alter the gender proportion of sexually undifferentiated juveniles to a preponderance of males, by increasing temperature during culture. De Bock and López Greco (2009) also showed higher temperatures can increase the proportion of males, which is a favourable characteristic in redclaw as males grow faster (Curtis and Jones 1995; Manor *et al*. 2002; Manor *et al*. 2004; Rodgers *et al*. 2006). It may be of economic value to more specifically identify the temperature to achieve the increased proportion of males.

A number of studies which have looked at temperature for culture of craylings and early stage juveniles have suggested an optimal water temperature of 27°C (Anson and Rouse 1996; Barki *et al*. 1997; Cortés-Jacinto *et al*. 2003; Campaña-Torres *et al*. 2005, 2008; Calvo *et al*. 2011; Calvo *et al*. 2013) whereas Garcia-Guerrero *et al*. (2003b) recommended an optimal temperature of 22°C – 25°C for aquaculture of early stage redclaw.

In summary, a nursery phase which would involve managed culture of redclaw juveniles from the crayling stage, will have additional cost. It is important to identify the optimal temperature to culture the juveniles to attain fastest possible growth without negatively impacting survival. There have been no published studies to date that address this issue and such knowledge may greatly enhance the prospects of the industry.

1.18 Habitat

Barki *et al.* (1997) suggest that cannibalism and predation are major causes of mortality during grow-out, and this may also apply to the nursery phase and has been shown in experiments where craylings were stocked to fiberglass tanks (Jones 1990b; Jones 1995b) and also with craylings stocked to experimental tanks in high densities with no refuge (Barki *et al*. 1997). Redclaw appear to be cannibalized whilst moulting (Barki *et al*. 1997), and there is evidence that redclaw avoid cannibalism by positioning themselves away from conspecifics in the shallow margins of earthen ponds or on top of habitat structures (Jones and Ruscoe 2001). Several studies have demonstrated the effectiveness of various materials in providing habitat for advanced juvenile and adult redclaw in ponds (Jones 1995d; Jones and Ruscoe 2001) that supported increased survival through presumed mitigation of conspecific predation. Jones and Ruscoe (2001) simulated macrophytes and Viau and Rodríguez (2009) used various-sized PVC pipes for habitat. Juvenile redclaw will take refuge in onion bags or mesh bundles which simulate macrophytes and which provide shelter and protection, and also decrease the density by providing larger areas of substrate.

There are no published data on effects of shelter / habitat on redclaw at the crayling stage, despite cannibalism being evident at this earliest free-living stage. Some candidates for suitable habitat for craylings include bundles of micro tubes (drinking straw diameter), which represent a scaled-down version of the bundles of pipes

commonly used for older redclaw in ponds (Jones and Ruscoe, 2001), onion bag mesh, or crevice type shelters as commonly used as an artificial habitat for capturing lobster pueruli (Priyambodo *et al*. 2015; Priyambodo *et al*. 2017). Specific research is warranted to understand crayling behaviour and the impact of provision of shelter on survival and growth in a nursery phase.

1.19 Density

Stocking density is another important factor which has a significant effect on redclaw production (Pinto and Rouse 1996; Jones and Ruscoe 2000; Naranjo-Paramo *et al.* 2004). Size at stocking and stocking density significantly impact yield for many crustacean species in aquaculture (Allan and Maguire 1992; Geddes *et al.* 1993; Daniels *et al*. 1995; Morrissy *et al*. 1995; Tidwell *et al*. 1999). For redclaw, it has been demonstrated that mean harvest weight is inversely related to original stocking density (Pinto and Rouse 1996; Jones and Ruscoe 2000; Naranjo-Paramo *et al*. 2004; Rodgers *et al*. 2006). Furthermore, yields are often directly related to density. Naranjo-Paramo *et al*. (2004) and Jones and Ruscoe (2000) found that mean food quotients, yields and economic returns significantly increased with increased stocking size and density. The juveniles in that study were of an advanced size (4.71 g and 16.89 g) and the stocking densities were relatively low (3, 9, 15 crayfish per m^2). There has been no specific examination of pond stocking density for craylings.

Size variability within a redclaw population can have a negative impact on smaller crayfish due to hierarchical dominance / subordinance behaviour (Karplus and Barki 2004). Karplus and Barki (2004) found the growth of small males was reduced by 50% when in contact with larger males attributed to increased inter-moult period and reduction in size increment per moult. Competition for food fed *ad libitum* when it was a defendable resource was found to contribute to this relationship, however the

addition of shelters to minimize interactions in nursery units resulted in an increase in juvenile weight (Karplus *et al.* 1995). This suggests that habitat may play an important role in attenuating the negative effects of high density (Jones and Ruscoe 2000). Conversely, Barki *et al.* (2006) used individual compartments to overcome social-dependent density limitations in a battery culture experiment. Male redclaw showed a lower growth rate when surrounded by neighbours, small crayfish surrounded by large neighbours also showed lower growth rates. Shelter alone may not be the solution to density growth rate suppression. The number and size of neighbours also have an effect. Naranjo-Paramo *et al*. (2004) conducted a nursery-stage study using 1.3 g juveniles in gravel-lined nursery ponds. They examined stocking densities of 5, 6, 8, 11 and 20 crayfish per m^2 grown for 80 days to see which densities could achieve a mean weight of at least 25 g redclaw in that time. The study found that densities of 11 crayfish per m^2 and lower achieved this, but at 20 per m^2, individual growth was significantly less.

Aquatic organisms in general display an inverse linear relationship between density and body size (Duarte *et al.* 1987). This is a fundamental regularity across all terrestrial and aquatic systems, showing that organisms use the space of 1/3 power of their body size (Duarte *et al*. 1987). Such a density effect however can be mitigated through the addition of habitat as it provides additional protection and space for the animals (Jones 1990b; Jones 1995c). Extremely low stocking density of animals may also have a negative impact if the carrying capacity of the environment is not fully used, leading to loss of production, higher per unit production cost and a loss of efficiency. For commercial aquaculture production, stocking density and its effects on overall production and mean harvest weight must be understood to achieve optimal economic outcomes. If redclaw craylings are to be cultured in a discrete nursery phase, research of stocking density effects will be necessary to determine optimal stocking practices.

Determining the highest stocking density possible with the provision of habitat at an optimal level will maximise production numbers, lower costs per unit and increase efficiency.

1.20 Summary

The development of artificial incubation 'hatchery' systems to produce redclaw crayfish craylings as seedstock for grow-out operations is new technology. The stimulus for such development was initially the desire to produce specific pathogen free (SPF) stock of a selectively bred 'domesticated' genetic strain (Stevenson *et al.* 2013). Prior to this new technology, production of redclaw juveniles was achieved by managing natural reproduction in ponds and sometimes tanks (Jones 1989). That 'traditional' approach still dominates the industry as the hatchery approach is developmental and not yet fully commercial. The disadvantages of the traditional approach are:

- in-pond breeding events which produce numerous extra cohorts and unknown densities (Sammy 1988; Jones *et al*. 2000; Karplus *et al.* 2003; Barki *et al*. 2006)
- low survival, likely 5 – 10% (Jones 1995b)
- inbreeding (McPhee *et al*. 2004)
- seasonal factors affect harvest success (Stevenson *et al*. 2013)
- inconsistent harvest due to inconsistent stocking (Stevenson *et al*. 2013)
- the effort and money spent raising seedstock in ponds is better spent on grow-out (Stevenson *et al.* 2013).

An intensive hatchery approach to crayling production provides significant advantages including:

- production of specific pathogen-free [SPF] animals (Stevenson *et al*. 2013)

- selectively breed for faster and more uniform growth (Stevenson *et al*. 2013)
- combat inbreeding depression (Stevenson *et al*. 2013)
- ability to produce stock all year round (Stevenson *et al*. 2013).

1.21 Future Research Directions

As with most aquaculture enterprises, production of high quality seedstock is a critical part of a successful operation. Inherent within this intensification process is the concept of minimising cost and introducing economies of scale to further enhance profitability. Effective hatchery technology will assist redclaw aquaculture to become more intensive and profitable. It is envisaged that a small number of dedicated hatcheries will provide the seedstock to the broader redclaw grow-out industry, in a fashion similar to that of most commercially successful aquaculture industries. For redclaw hatchery technology to become fully commercial, greater consistency of survival through the incubation phase is required, along with standard operating procedures for the subsequent nursing of the craylings.

The research required includes formulation of a specific crayling nursery diet, optimization of feeding husbandry (feeding frequency, ration, feed delivery, feed form) and management protocols that maximize survival and quality of the juveniles produced (Garcia-Guerrero *et al.* 2003a). The time that craylings are held in a nursery period and which temperature, type and quantity of habitat and stocking density need to be established.

The diet itself must contain protein, lipids and carbohydrates in the most appropriate ratio, at a specific energy and digestibility value, and be delivered within a water-stable pellet of a size ideal for crayling mouth parts. The onset of the ontogenetic change from crayling diet to the next stage must be identified. This will require a change in protein, lipid, and carbohydrate ratio in the feed, as evidenced by the changes in

amino acid and lipid content within the animal itself at this life stage (Garcia-Guerrero *et al*. 2003a).

The optimal period to hold the craylings to maximize survival and growth needs to be explored. The duration of the nursery phase will be a balance between cost and achieving a juvenile size that is optimal for pond stocking in relation to subsequent growth and survival through grow-out.

The most advantageous temperature to culture craylings for quickest growth, and highest survival in a nursery phase is yet to be determined. Higher temperature may increase growth rate but at the expense of survival (King 1994; García-Ulloa *et al*. 2003), while low temperature will support slow growth but higher survival rates (King 1994; García-Ulloa *et al.* 2003). The intersection of these two factors to determine the optimal temperature has yet to be established.

A nursery phase which provides the best dietary parameters and temperature may help moderate the problem of cannibalism. The reasonably poor survival shown in grow-out operations using craylings may be mitigated by introducing more advanced juveniles. However, since it has not yet been established why there appears to be such a high level of cannibalism, other possible factors need to be explored. The provision of structure within a nursery period would give the animals somewhere to hide whilst going through ecdysis, as very small crayfish moult frequently (Jones and Ruscoe 2000) and this is when they are preyed upon by conspecifics (Ghanawi and Saoud 2012). At the nursery stage, experiments could be conducted to determine the optimal habitat. This would also assist in lowering the effective stocking density and allowing more animals to be held.

A nursery phase immediately following the hatchery production would nurture the crayfish from crayling to an advanced juvenile and provide potential positive

benefits to the redclaw aquaculture industry. These potential benefits include a reduction in variability of survival through grow-out, which could reduce the risk to the farmer of unknown crop size. Hatchery / nursery operations can remove the technical side of producing seedstock from the average farmer so they can concentrate on the grow-out. This is a typical scenario for more mature aquaculture industries such as those for Barramundi (*Lates calcarifer*) where once the research and development phase is over many hatcheries are set up for ease of grow-out, more predictable yields, and lower transport costs.

The final process in the intensification of a combined hatchery / nursery production phase for redclaw would be to gather together the results from all the research and produce a best practice management protocol for producing juveniles for grow-out operations. It is likely that a nursery phase for redclaw will provide more surety, interest and investment in what is a potentially highly profitable industry, producing high value food, employment and rural income in Australia and subtropical and tropical regions elsewhere in the world.

1.23 Literature Cited

ABDU U, HARPAZ S AND KARPLUS H (1997). Culture of the Australian Red-Claw Crayfish (*Cherax quadricarinatus*) in Israel. *Israeli Journal of Aquaculture—Bamidgeh* 49(4):222-229. doi: not available.

ACKEFORS H (1994). Recent progress in Australian crayfish culture. *World Aquaculture* 25(4):14-19. doi: not available.

ACKEFORS HEG (2000). Freshwater crayfish farming technology in the 1990s: a European and global perspective. *Fish and Fisheries* 1(4):337-359. doi: 10.1046/j.1467-2979.2000.00023.x.

AHAMED ALI S (1993). Evaluation of different carbohydrates in the diet of the prawn *Penaeus indicus*. *Journal of Aquaculture in the Tropics* 8(1):13-23. doi: not available.

ALLAN GL AND MAGUIRE GB (1992). Effects of stocking density on production of *Penaeus monodon* Fabricius in model farming ponds. *Aquaculture* 107(1):49-66. doi: doi.org/10.1016/0044-8486(92)90049-Q.

ANSON KJ AND ROUSE DB (1994). Effects of salinity on hatching and post-hatch survival of the Australian red claw crayfish *Cherax quadricarinatus*. *Journal of the World Aquaculture Society* 25(2):277-280. doi: 10.1111/j.1749-7345.1994.tb00191.x.

ANSON KJ AND ROUSE DB (1996). Evaluation of several commercial feeds and Crustacean Reference Diet for juvenile Australian Red Claw crayfish, *Cherax quadricarinatus*. *Journal of Applied Aquaculture* 6(1):65-76. doi: 10.1300/J028v06n01_06.

ARREDONDO-FIGUEROA JL, PONCE-PALAFOX JT, SHIRAI-MATSUMOTO K, PÉREZ-ZAVALETA Á, BARRIGA-SOSA IDLÁ AND RUIZ LUNA A (2013). Effects of including shrimp protein hydrolysate in practical diets on the growth and survival of redclaw crayfish hatchlings *Cherax quadricarinatus* (Von Martens, 1868). *Aquaculture Research* 44(6):966-973. doi: 10.1111/j.1365-2109.2012.03102.x.

ASGARI L (2004). Broodstock nutrition of Australian Red Claw Crayfish *Cherax quadricarinatus* (Von Martens). Agriculture. Universiti Putra Malaysia.

BARKER PL AND GIBSON R (1977). Observations on the feeding mechanism, structure of the gut, and digestive physiology of the European lobster *Homarus gammarus* (L.)(Decapoda: Nephropidae). *Journal of Experimental Marine Biology and Ecology* 26(3):297-324. doi: https://doi.org/10.1016/0022-0981(77)90089-2.

BARKI A AND KARPLUS I (1999). Behavioural assay for receptivity in female *Cherax quadricarinatus*: does she show? *Freshwater Crayfish* 12:26. doi: not available.

BARKI A AND KARPLUS I (2000). Crowding female red claw crayfish, *Cherax quadricarinatus*, under small-tanks hatchery conditions: what is the limit? *Aquaculture* 181(3-4):235-240. doi: https://doi.org/10.1016/S0044-8486(99)00235-5.

BARKI A, KARPLUS I, MANOR R, PARNES S, AFLALO E AND SAGI A (2006). Growth of redclaw crayfish (*Cherax quadricarinatus*) in a three-dimensional compartments system: Does a neighbor matter? *Aquaculture* 252(2-4):348-355. doi: 10.1016/j.aquaculture.2005.07.012.

BARKI A, LEVI T, SHREM A AND KARPLUS I (1997). Ration and spatial distribution of feed affect survival, growth, and competition in juvenile red-claw crayfish, *Cherax quadricarinatus*, reared in the laboratory. *Aquaculture* 148(1997):169-177. doi: https://doi.org/10.1016/S0044-8486(96)01418-4.

BITOMSKY J (2008). Scoping Report Redclaw Industry Development. Kleinhardt Business Consultants, Cairns.

BROWN PB, WETZEL II JE, SPACIE A AND KONOPKA A (1992). Evaluation of naturally occurring organisms as food for juvenile crayfish *Procambarus clarkii*. *Journal of the World Aquaculture Society* 23:211-216. doi: doi/pdf/10.1111/j.1749-7345.1992.tb00771.x.

BUGNOT AB AND LOPEZ GRECO LS (2009). Structural alterations in the male reproductive system of the freshwater crayfish, *Cherax quadricarinatus* (Decapoda, Parastacidae). *J Invertebr Pathol* 102(2):160-166. doi: 10.1016/j.jip.2009.08.004.

CALVO NS, STUMPF L, PIETROKOVSKY S AND LÓPEZ GRECO LS (2011). Early and late effects of feed restriction on survival, growth and hepatopancreas structure in juveniles of the red claw crayfish *Cherax quadricarinatus*. *Aquaculture* 319(3-4):355-362. doi: 10.1016/j.aquaculture.2011.06.033.

CALVO NS, STUMPF L, SACRISTÁN HJ AND LÓPEZ GRECO LS (2013). Energetic reserves and digestive enzyme activities in juveniles of the red claw crayfish *Cherax quadricarinatus* nearby the point-of-no-return. *Aquaculture* 416-417:85-91. doi: 10.1016/j.aquaculture.2013.08.017.

CAMPAÑA T (2001). Digestibility of vegetal and animal ingredients and diets for juvenile and pre-adult redclaw *Cherax quadricarinatus*. CIBNOR, SC La Paz, BCS, Mexico.

CAMPANA-TORRES A, MARTINEZ-CORDOVA LR, VILLARREAL-COLMENARES H AND CIVERA-CERECEDO R (2006). Carbohydrate and lipid digestibility of animal and vegetal ingredients and diets for juvenile Australian redclaw crayfish, *Cherax quadricarinatus*. *Aquaculture Nutrition* 12(2):103-109. doi: https://doi.org/10.1111/j.1365-2095.2006.00388.x.

CAMPAÑA-TORRES A, MARTINEZ-CORDOVA LR, VILLARREAL-COLMENARES H AND CIVERA-CERECEDO R (2005). In vivo dry matter and protein digestibility of three plant-derived and four animal-derived feedstuffs and diets for juvenile Australian redclaw, *Cherax quadricarinatus*. *Aquaculture* 250(3-4):748-754. doi: 10.1016/j.aquaculture.2005.02.058.

CAMPAÑA-TORRES A, MARTINEZ-CORDOVA LR, VILLARREAL-COLMENARES H AND CIVERA-CERECEDO R (2008). Carbohydrate and lipid digestibility of animal and vegetal ingredients and diets for the pre-adult redclaw crayfish, *Cherax quadricarinatus* (von Martens). *Aquaculture Research* 39(10):1115-1121. doi: 10.1111/j.1365-2109.2008.01980.x.

CELADA JD, CARRAL JM, GAUDIOSO VR, TEMIÑO C AND FERNÁNDEZ R (1989). Response of juvenile freshwater crayfish (*Pacifastacus leniusculus* Dana) to several fresh and artificially compounded diets. *Aquaculture* 76(1-2):67-78. doi: https://doi.org/10.1016/0044-8486(89)90252-4.

CORTÉS-JACINTO E, CAMPA-CÓRDOVA ÁI, ASCENCIO F, VILLARREAL-COLMENARES H AND HOLGUÍN-PEÑA RJ (2009). The effect of protein and energy levels in diet on the antioxidant activity of juvenile redclaw *Cherax quadricarinatus* (von Martens, 1868) Efecto del nivel de proteína y energía en la actividad antioxidante de juveniles de langosta de agua dulce *Cherax quadricarinatus* (von Martens, 1868). *Hidrobiológica* 19(2):77-83. doi: not available.

CORTÉS-JACINTO E, VILLARREAL-COLMENARES H, CIVERA-CERECEDO R AND CRUZ-SUÁREZ L (2004). Studies on the nutrition of the freshwater crayfish *Cherax quadricarinatus* (von Martens): effect of the dietary protein level on growth of juveniles and preadults. *Freshwater Crayfish* 14:70-80. doi: not available.

CORTES-JACINTO E, VILLARREAL-COLMENARES H, CRUZ-SUAREZ LE, CIVERA-CERECEDO R, NOLASCO-SORIA H AND HERNANDEZ-LLAMAS A (2005). Effect of different dietary protein and lipid levels on growth and survival of juvenile Australian redclaw crayfish, *Cherax quadricarinatus* (von Martens). *Aquaculture Nutrition* 11(4):283-291. doi: https://doi.org/10.1111/j.1365-2095.2005.00353.x.

CORTÉS-JACINTO E, VILLARREAL-COLMENARES H, CIVERA-CERECEDO R AND MARTÍNEZ-CÓRDOVA R (2003). Effect of dietary protein level on growth and survival of juvenile freshwater crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *Aquaculture Nutrition* 9(4):207-213. doi: https://doi.org/10.1046/j.1365-2095.2003.00241.x.

CORTÉS-JACINTO E, VILLARREAL-COLMENARES H, CIVERA-CERECEDO R AND NARANJO-PÁRAMO J (2004). Effect of dietary protein level on the growth and survival of pre-adult freshwater crayfish *Cherax quadricarinatus* (von Martens) in monosex culture. *Aquaculture Research* 35(1):71-79. doi: https://doi.org/10.1111/j.1365-2109.2004.00988.x.

CURTIS MC AND JONES CM (1995). Observations on monosex culture of redclaw crayfish *Cherax quadricarinatus* von Martens (Decapoda: Parastacidae) in earthen ponds. *Journal of the World Aquaculture Society* 26(2):154-159. doi:

D'ABRAMO LR, WRIGHT JS, WRIGHT KH, BORDNER CE AND CONKLIN DE (1985). Sterol requirement of cultured juvenile crayfish, *Pacifastacus leniusculus*. *Aquaculture* 49(3):245-255. doi: https://doi.org/10.1016/0044-8486(85)90083-3.

D'ABRAMO LR AND ROBINSON EH (1989). Nutrition of crayfish. *Reviews in Aquatic Sciences* 1(4):711-728. doi: not available.

DALL W, HILL BJ, ROTHLISBERG PC AND SHARPLES DJ (1991). The biology of the Penaeidae. *Advances in Marine Biology* 27:489. doi: https://doi.org/10.1016/S0065-2881(08)60166-2.

DAMMANNAGODA LK, PAVASOVIC A, HURWOOD DA AND MATHER PB (2015). Effects of soluble dietary cellulose on specific growth rate, survival and digestive enzyme activities in three freshwater crayfish (*Cherax*) species. *Aquaculture Research* 46(3):626-636. doi: 10.1111/are.12209.

DANIELS WH, D'ABRAMO LR, FONDREN MW AND DURANT MD (1995). Effects of stocking density and feed on pond production characteristics and revenue of harvested freshwater prawns *Macrobrachium rosenbergii* stocked as size-graded juveniles. *Journal of the World Aquaculture Society* 26(1):38-47. doi: https://doi.org/10.1111/j.1749-7345.1995.tb00207.x.

DE BOCK MS AND LÓPEZ GRECO LS (2009). Sex reversal and growth performance in juvenile females of the freshwater crayfish *Cherax quadricarinatus* (Parastacidae): effect of increasing temperature and androgenic gland extract in the diet. *Aquaculture International* 18(2):231-243. doi: 10.1007/s10499-008-9239-x.

DOUPÉ, RG, MORGAN, DL, GILL, HS, & ROWLAND, AJ (2004). Introduction of redclaw crayfish *Cherax quadricarinatus* (von Martens) to Lake Kununurra, Ord River, Western Australia: prospects for a 'yabby' in the Kimberley. *Journal of the Royal Society of Western Australia*, *87*(4), 187-191.

DUARTE CM, AGUSTI S AND PETERS H (1987). An upper limit to the abundance of aquatic organisms. *Oecologia* 74(2):272-276. doi: https://doi.org/ 10.1007/BF00379370.

FAO (2016). The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Pp.200. FAO, Rome.

FAO (2017). *Cherax quadricarinatus* (von Martens, 1868). Cultured species information program. *In:* [online] FAO Fisheries and Aquaculture Department [online]

FIGUEIREDO MSRB AND ANDERSON AJ (2003). Ontogenetic changes in digestive proteases and carbohydrases from the Australian freshwater crayfish, redclaw *Cherax quadricarinatus* (Crustacea, Decapoda, Parastacidae). *Aquaculture Research* 34(13):1235-1239. doi: not available.

FIGUEIREDO MSRB, KRICKER JA AND ANDERSON AJ (2001). Digestive Enzyme Activities in the Alimentary Tract of Redclaw Crayfish, *Cherax quadricarinatus* (Decapoda: Parastacidae). *Journal of Crustacean Biology* 21(2):334-344. doi: https://doi.org/10.1163/20021975-99990133.

FLETCHER A AND WARBURTON K (1997). Consumption of fresh and decomposed duckweed *Spirodela* sp. by redclaw crayfish, *Cherax quadricarinatus* (von Martens). *Aquaculture Research* 28(5):379-382. doi: https://doi.org/10.1046/j.1365-2109.1997.t01-1-00808.x.

FRDC (2010). Working together: the national fishing and aquaculture RD & E strategy 2010. Commonwealth of Australia, Canberra.

GARCÍA-GUERRERO M, HERNÁNDEZ-SANDOVAL P, ORDUÑA-ROJAS J AND CORTÉS-JACINTO E (2013). Effect of temperature on weight increase, survival, and thermal preference of juvenile redclaw crayfish *Cherax quadricarinatus*. *Hidrobiológica* 23(1):73-81. doi: not available.

GARCIA-GUERRERO M, RACOTTA IS, RODRÍGUEZ-JARAMILLO C, VILLARREAL H AND CORTES-JACINTO E (2003a). Energy storage during the transition from endogenous to exogenous feeding in Australian redclaw crayfish *Cherax quadricarinatus* (Von Martens, 1898). *Invertebrate Reproduction and Development* 44(2-3):101-106. doi: https://doi.org/10.1080/

07924259.2003.9652560.

GARCÍA-GUERRERO M, VILLARREAL H AND RACOTTA IS (2003b). Effect of temperature on lipids, proteins, and carbohydrates levels during development from egg extrusion to juvenile stage of *Cherax quadricarinatus* (Decapoda: Parastacidae). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 135(1):147-154. doi: 10.1016/s1095-6433(02)00354-9.

GARCÍA-ULLOA GM, LÓPEZ-CHAVARÍN HM, RODRÍGUEZ-GONZÁLEZ H AND VILLARREAL-COLMENARES H (2003). Growth of redclaw crayfish *Cherax quadricarinatus* (Von Martens 1868)(Decapoda: Parastacidae) juveniles fed isoproteic diets with partial or total substitution of fish meal by soya bean meal: preliminary study. *Aquaculture Nutrition* 9(1):25-31. doi: https://doi.org/10.1046/j.1365-2095.2003.00224.x.

GARZA DE YTA A (2009). Hatchery, nursery, nutrition and stock evaluation of redclaw crayfish *Cherax quadricarinatus*. Pp. 135. Auburn University.

GARZA DE YTA A, DAVIS DA, ROUSE DB, SAOUD IP AND GHANAWI J (2011). Effects of Stargrass Hay Supplementation on Growth and Survival of Juvenile Redclaw Crayfish *Cherax quadricarinatus*. *North American Journal of Aquaculture* 73(4):484-488. doi: 10.1080/15222055.2011.635244.

GEDDES MC, SMALLRIDGE M AND CLARKE S (1993). The effect of stocking density, food type and shelters on survival and growth of the Australian freshwater crayfish, *Cherax destructor*, in experimental ponds. *Freshwater Crayfish* 9:57-69. doi: not available.

GHANAWI J AND SAOUD IP (2012). Molting, reproductive biology, and hatchery management of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868). *Aquaculture* 358-359:183-195. doi: https://doi.org/10.1016/j.aquaculture.2012.06.019.

GUILLAUME J (1997). Protein and amino acids. Pp. 26-50 *In:* D'Abramo LR, Conklin DE and Akiyama DM, eds.). Crustacean Nutrition. Adavances in World Aquaculture. World Aquaculture Society, Baton Rouge, Louisiana.

GUILLAUME J AND CHOUBERT G (2001). Digestive physiology and nutrient digestibility in fishes. Pp. 27-56 *In:* Guillaume J, Kaushik S, Bergot P and Metailler R, (eds.). Nutrition and Feeding of fFsh and Crustaceans. Springer Praxis United Kingdom.

GUTIÉRREZ M AND RODRÍGUEZ E (2010). Effect of protein source on growth of early juvenile redclaw crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae). *Freshwater Crayfish* 17:23-29. doi: not available.

HAUBROCK, PJ, OFICIALDEGUI, FJ, ZENG, Y, PATOKA, J, YEO, DC, & KOUBA, A (2021). The redclaw crayfish: A prominent aquaculture species with invasive potential in tropical and subtropical biodiversity hotspots. *Reviews in Aquaculture*, 13(3), 1488-1530.

HENRIKSSON PJG, GUINÉE JB, KLEIJN R AND DE SNOO GR (2012). Life cycle assessment of aquaculture systems—a review of methodologies. *The International Journal of Life Cycle Assessment* 17(3):304-313. doi: 10.1007/s11367-011-0369-4.

HERNANDEZ PV, OLVERA-NOVOA MA AND ROUSE DB (2004). Effect of dietary cholesterol on growth and survival of juvenile redclaw crayfish *Cherax quadricarinatus* under laboratory conditions. *Aquaculture* 236(1-4):405-411. doi: https://doi.org/10.1016/S0044-8486(03)00135-2.

HERNANDEZ-VERGARA MP, ROUSE DB, OLVERA-NOVOA MA AND DAVIS DA (2003). Effects of dietary lipid level and source on growth and proximate composition of juvenile redclaw (*Cherax quadricarinatus*) reared under semi-intensive culture conditions. *Aquaculture* 223(1-4):107-115. doi: not available.

HIGLEY LG, PEDIGO LP AND OSTLIE KR (1986). DEGDAY: a program for calculating degree-days, and assumptions behind the degree-day approach. *Environmental Entomology* 15(5):999-1016. doi: https://doi.org/10.1093/ee/15.5.999.

HSIEH, CY, HUANG, CW, & PAN, YC (2016). Crayfish plague Aphanomyces astaci detected in redclaw crayfish, Cherax quadricarinatus in Taiwan. *Journal of invertebrate pathology*, 136, 117-123.

JOAQUÍ P AND MONTES C (2001). Bioenergetics of juveniles of red swamp crayfish (*Procambarus clarkii*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 130(1):29-38. doi: https://doi.org/10.1016/S1095-6433(01)00358-0.

JONES CM (1989). The Biology and Aquaculture Potential of *Cherax quadricarinatus.* Final Report for Project "The Assessment of *Cherax quadricarinatus* as a Candidate for Aquaculture. Reserve Bank of Australia, Rural Credits Development Fund, Project QDPI/8860. Pp. 131. Department of Primary Industries, Queensland, Walkamin, Australia.

JONES CM (1990a). Biology and aquaculture potential of the tropical Australian freshwater crayfish (*Cherax quadricarinatus*). Pp. 19. Abstracts, Eighth International Symposium of the International Association of Astacology, Baton Rouge, USA, April 22-26, 1990. International Association of Astacology, Baton Rouge.

JONES CM (1990b). The Biology and Aquaculture Potential of the Tropical Freshwater Crayfish, *Cherax quadricarinatus.* QI90028. Department of Primary Industries, Queensland, Brisbane, Australia.

JONES CM (1995a). Evaluation of six diets fed to redclaw, *Cherax quadricarinatus* (von Martens), held in pond enclosures. *Freshwater Crayfish* 10:469-479. doi: not available.

JONES CM (1995b). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens)(Decapoda, Parastacidae) I. Development of hatchery and nursery procedures. *Aquaculture* 138(1995):221-238. doi: https://doi.org/10.1016/0044-8486(95)00068-2.

JONES CM (1995c). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens)(Decapoda, Parastacidae) II. Juvenile nutrition and habitat. *Aquaculture* 138(1995):239-245. doi: https://doi.org/10.1016/0044-8486(95)00069-0.

JONES CM (1995d). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens)(Decapoda, Parastacidae) III. Managed pond production trials. *Aquaculture* 138(1995):247-255. doi: 10.1016/0044-8486(95)00067-4.

JONES CM (1995e). Salinity tolerance of the tropical freshwater crayfish, *Cherax quadricarinatus* (von Martens)(Decapoda, Parastacidae). *Freshwater Crayfish* 8:399-409. doi: not available.

JONES CM AND CURTIS MC (1994). Redclaw Farming. Pp. 69. Redclaw Farming Workshops, Feb.12-17, 1994, Walkamin, Rockhampton, Nambour. Queensland Department of Primary Industries.

JONES CM, MCPHEE CP AND RUSCOE IM (2000). A review of genetic improvement in growth rate in redclaw crayfish *Cherax quadricarinatus* (von Martens) (Decapoda: Parastacidae). *Aquaculture Research* 31(1):61-67. doi: 10.1046/j.1365-2109.2000.00430.x.

JONES CM AND RUSCOE IM (1996). Evaluation of six diets fed to redclaw, *Cherax quadricarinatus* (von Martens), (Decapoda: Parastacidae), under laboratory conditions. Pp. 19-30 Production Technology for Redclaw Crayfish (*Cherax quadricarinatus*). Final Report, Project 92/119, Fisheries Research and Development Corporation. Department of Primary Industries, Queensland, Walkamin.

JONES CM AND RUSCOE IM (2000). Assessment of stocking size and density in the production of redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda: Parastacidae), cultured under earthen pond conditions. *Aquaculture* 189(1-2):63-71. doi: 10.1016/S0044-8486(00)00359-8.

JONES CM AND RUSCOE IM (2001). Assessment of five shelter types in the production of redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae) under earthen pond conditions. *Journal of the World Aquaculture Society* 32(1):41-52. doi: 10.1111/j.1749-7345.2001.tb00920.x.

JONES CM AND RUSCOE IM (2002). Biological and aquaculture characteristics of five stocks of redclaw, *Cherax quadricarinatus* (von Martens)(Decapoda: Parastacidae) from northern Queensland, Australia Freshwater Crayfish. *Freshw Crayfish* 13:115-135. doi: not available.

JONES CM AND VALVERDE C (2001). Development of Mass Production Hatchery Technology for the Redclaw Crayfish, *Cherax quadricarinatus. Freshw Crayfish* in press.

KARPLUS I AND BARKI A (2004). Social control of growth in the redclaw crayfish, *Cherax quadricarinatus*: testing the sensory modalities involved. *Aquaculture* 242(1-4):321-333. doi: 10.1016/j.aquaculture.2004.08.041.

KARPLUS I, BARKI A, LEVI T, HULATA G AND HARPAZ S (1995). Effect of kinship and shelters on growth and survival of juvenile Australian redclaw crayfish (*Cherax quadricarinatus*). *Freshwater Crayfish* 10:494-505. doi: not available.

KARPLUS I, GIDEON H AND BARKI A (2003). Shifting the natural spring–summer breeding season of the Australian freshwater crayfish *Cherax quadricarinatus* into the winter by environmental manipulations. *Aquaculture* 220(1-4):277-286. doi: 10.1016/s0044-8486(02)00225-9.

KING CR (1994). Growth and survival of red claw crayfish hatchlings (*Cherax quadricarinatus* von Martens) in relation to temperature, with comments on the relative suitability of *Cherax quadricarinatus* and *Cherax destructor* for culture

in Queensland. *Aquaculture* 122:75-80. doi: https://doi.org/10.1016/0044-8486(94)90335-2.

KONDOS A (1990). Supplementary feed essential for crayfish. *Australian Fisheries* 49(11):28-30. doi: not available.

LAVALLI KL AND FACTOR JR (1992). Functional morphology of the mouthparts of juvenile lobsters, *Homarus americanus* (Decapoda: Nephropidae), and comparison with the larval stages. *Journal of Crustacean Biology* 12(3):467-510. doi: https://doi.org/10.2307/1549041.

LAVALLI KL AND FACTOR JR (1995). The feeding appendages. Pp. 349-393 *In:* Factor JR, (ed. Biology of the Lobster *Homarus americanus*. Academic press, San Diego.

LAWRENCE C AND JONES C (2002). Chapter 17. *Cherax*. Pp. 635-670 *In:* Holdich DM, (ed. Biology of freshwater crayfish. Blackwell Science Ltd, Oxford.

LEVI T, BARKI A, HULATA G AND KARPLUS I (1999). Mother-offspring relationships in the red-claw crayfish *Cherax quadricarinatus*. *Journal of Crustacean Biology* 19(3):477-484. doi: doi:10.2307/1549256.

LI JY, GUO ZL, GAN XH, WANG DL, ZHANG MF AND ZHAO YL (2011). Effect of different dietary lipid sources on growth and gonad maturation of pre-adult female *Cherax quadricarinatus* (von Martens). *Aquaculture Nutrition* 17(4):e853-e860. doi: 10.1111/j.1365-2095.2011.00852.x.

LODGE D AND HILL A (1994). Factors governing species composition, population size and productivity of cool-water cray-fishes. *Nordic Journal of Freshwater Research (Sweden)*. doi: not available.

LOPEZ-LOPEZ S, NOLASCO H, VILLARREAL-COLMENARES H AND CIVERA-CERECEDO R (2005). Digestive enzyme response to supplemental ingredients in practical diets for juvenile freshwater crayfish *Cherax quadricarinatus*. *Aquaculture Nutrition* 11(2):79-85. doi: https://doi.org/10.1111/j.1365-2095.2004.00305.x.

LOVETT DL AND FELDER DL (1990). Ontogenetic change in digestive enzyme activity of larval and postlarval white shrimp *Penaeus setiferus* (Crustacea, Decapoda, Penaeidae). *The Biological Bulletin* 178(2):144-159. doi: https://doi.org/10.2307/1541973.

LOYA-JAVELLANA GN AND FIELDER DR (1997). Developmental trends in the mouthparts during growth from juvenile to adult of the tropical freshwater crayfish, *Cherax quadricarinatus* von Martens, 1868 (Decapoda: Parastacidae). *Invertebrate reproduction & development* 32(2):167-175. doi: 10.1080/07924259.1997.9672619.

LOYA-JAVELLANA GN, FIELDER DR AND THORNE MJ (1993). Food choice by free-living stages of the tropical freshwater crayfish, *Cherax quadricarinatus* (Parastacidae: Decapoda). *Aquaculture* 118(3):299-308. doi: https://doi.org/10.1016/0044-8486(93)90464-A.

MANOR R, AFLALO ED, SEGALL C, WEIL S, AZULAY D, VENTURA T AND SAGI A (2004). Androgenic gland implantation promotes growth and inhibits vitellogenesis in *Cherax quadricarinatus* females held in individual compartments. *Invertebrate reproduction & development* 45(2):151-159. doi: 10.1080/07924259.2004.9652584.

MANOR R, SEGEV R, LEIBOVITZ MP, AFLALO ED AND SAGI A (2002). Intensification of redclaw crayfish *Cherax quadricarinatus* culture: II. Growout in a separate cell system. *Aquacultural Engineering* 26(4):263-276. doi: https://doi.org/10.1016/S0144-8609(02)00035-3.

MASON J (1975). Crayfish production in a small woodland stream. *Freshwater Crayfish* 2:449-479. doi: not available.

MASSER M AND ROUSE D (1993). Production of Australian red claw crayfish. Alabama Cooperative Extension Service, USA.

MASSER MP AND ROUSE BD (1997). Australian Red Claw Crayfish. SRAC Publication No.244. The Alabama Cooperative Extension Service, USA.

MCCLAIN WR, NEILL WH AND GATLIN DM (1992). Nutrient profiles of green and decomposed rice-forages and their utilization by juvenile crayfish (*Procambarus clarkii*). *Aquaculture* 101(3-4):251-265. doi: https://doi.org/10.1016/0044-8486(92)90029-K.

MCPHEE CP, JONES CM AND SHANKS SA (2004). Selection for increased weight at nine months in Redclaw crayfish (*Cherax quadricarinatus*). *Aquaculture* 237(1-4):131-140. doi: 10.1016/j.aquaculture.2004.05.008.

MEADE ME AND WATTS SA (1995). Weight gain and survival of juvenile Australian crayfish *Cherax quadricarinatus* fed formulated feeds. *Journal of the World Aquaculture Society* 26(4):469-474. doi:

MEDLEY PB, NELSON RG, HATCH LU, ROUSE DB AND PINTO GF (1994). Economic feasibility and risk analysis of Australian red claw crayfish *Cherax quadricarinatus* aquaculture in the southeastern United States. *Journal of the World Aquaculture Society* 25(1):135-146. doi: https://doi-org.elibrary.jcu.edu.au/10.1111/j.1749-7345.1994.tb00813.x.

METTS LS, THOMPSON KR, XIONG Y, KONG B, WEBSTER CD AND BRADY Y (2007). Use of alfalfa hay, compared to feeding practical diets containing two protein levels, on growth, survival, body composition, and processing traits of Australian red claw crayfish, *Cherax quadricarinatus*, grown in ponds. *Journal of the World Aquaculture Society* 38(2):218-230. doi: https://doi.org/10.1111/j.1749-7345.2007.00091.x.

MITCHELL BD AND COLLINS R (1989). Development of field-scale intensive culture techniques for the commercial production of the yabbie (*Cherax destructor/albidus*). Centre for Aquatic Science, Warrnambool Institute of Advanced Education, Warrnambool, Victoria.

MOMOT WT (1995). Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science* 3(1):33-63. doi: 10.1080/10641269509388566.

MORRISSY NM, BIRD C AND CASSELLS G (1995). Density-dependent growth of cultured marron, *Cherax tenuimaus* (Smith 1912). *Freshwater Crayfish* 10:560-568. doi: not available.

MSANGI S, KOBAYASHI M, BATKA M, VANNUCCINI S, DEY M AND ANDERSON J (2013). Fish to 2030: prospects for fisheries and aquaculture. *World Bank Report*(83177-GLB). doi: not available.

MUZINIC LA, THOMPSON KR, MORRIS A, WEBSTER CD, ROUSE DB AND MANOMAITIS L (2004). Partial and total replacement of fish meal with soybean meal and brewer's grains with yeast in practical diets for Australian red claw crayfish *Cherax quadricarinatus*. *Aquaculture* 230(1-4):359-376. doi: https://doi.org/10.1016/S0044-8486(03)00420-4.

NARANJO-PARAMO J, HERNANDEZ-LLAMAS A AND VILLARREAL H (2004). Effect of stocking density on growth, survival and yield of juvenile redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae) in gravel-lined commercial nursery ponds. *Aquaculture* 242(1-4):197-206. doi: https://doi.org/10.1016/j.aquaculture.2004.05.017.

NYSTROM P (2002). Ecology *In:* Holdich DM, (ed.) Biology of freshwater crayfish. Blackwell, London.

O'SULLIVAN D, FIELDER D AND JONES C (2003). Chapter 20. Freshwater Crustaceans. Pp. 420-442 *In:* Lucas JS and Southgate PC, (eds.). Aquaculture: Farming Aquatic Animals and Plants. Blackwell Publishing, Oxford.

PARNES S AND SAGI A (2002). Intensification of redclaw crayfish *Cherax quadricarinatus* culture: I. Hatchery and nursery system. *Aquacultural Engineering* 26(4):251-262. doi: https://doi.org/10.1016/S0144-8609(02)00034-1.

PARVATHY K (1971). Glycogen storage in relation to the moult cycle in the two crustaceans *Emerita asiatica* and *Ligia exotica*. *Marine Biology* 10(1):82-86. doi: https://doi.org/10.1007/BF02026770.

PAVASOVIC A, ANDERSON AJ, MATHER PB AND RICHARDSON NA (2007a). Effect of a variety of animal, plant and single cell-based feed ingredients on diet digestibility and digestive enzyme activity in redclaw crayfish, *Cherax quadricarinatus* (Von Martens 1868). *Aquaculture* 272(1–4):564-572. doi: https://doi.org/10.1016/j.aquaculture.2007.08.027.

PAVASOVIC A, ANDERSON AJ, MATHER PB AND RICHARDSON NA (2007b). Influence of dietary protein on digestive enzyme activity, growth and tail muscle composition in redclaw crayfish, *Cherax quadricarinatus* (von Martens). *Aquaculture Research* 38(6):644-652. doi: 10.1111/j.1365-2109.2007.01708.x.

PAVASOVIC A, RICHARDSON NA, MATHER PB AND ANDERSON AJ (2006). Influence of insoluble dietary cellulose on digestive enzyme activity, feed digestibility and survival in the red claw crayfish, *Cherax quadricarinatus* (von Martens). *Aquaculture Research* 37(1):25-32. doi: https://doi.org/10.1111/j.1365-2109.2005.01389.x.

PILLAY TVR (1990). Aquaculture: principles and practices. Fishing News Books.

PINTO GF AND ROUSE DB (1996). Growth and survival of the Australian red claw crayfish *Cherax quadricarinatus* at three densities in earthen ponds. *Journal of the World Aquaculture Society* 27(2):187-193. doi: https://doi.org/10.1111/j.1749-7345.1996.tb00268.x.

PIROZZI I, JOYCE M, CLAY S, OWENS L, VILLACIS CONRADO D, HUU LE H, CHAU NGUYEN M, PICHETTE A AND STRIGHT T (2015). Boosting redclaw industry productivity with improved nutrition & feed management. Final Report for

RIRDC Project No. PRJ-008536. Pp. 123 *In:* Corporation AGRIRaD, (ed.) Australian Government, Canberra.

PRIYAMBODO B, JONES C AND SAMMUT J (2015). The effect of trap type and water depth on puerulus settlement in the spiny lobster aquaculture industry in Indonesia. *Aquaculture* 442:132-137. doi: http://dx.doi.org/10.1016/j.aquaculture.2015.02.037.

PRIYAMBODO B, JONES CM AND SAMMUT J (2017). Improved collector design for the capture of tropical spiny lobster, *Panulirus homarus* and *P. ornatus* (Decapoda: Palinuridae), pueruli in Lombok, Indonesia. *Aquaculture* 479:321-332. doi: https://doi.org/10.1016/j.aquaculture.2017.05.033.

QUEENSLAND GOVERNMENT (2007). Redclaw crayfish information package. Pp. 92 Queensland Government, Brisbane.

QUEENSLAND GOVERNMENT (2015). Ross Lobegeiger Report to Farmers. Aquaculture production summary for Queensland 2014 -15. Pp. 11 Queensland Government, Brisbane.

QUEENSLAND GOVERNMENT (2016). Ross Lobegeiger Report to Farmers. Aquaculture production summary for Queensland 2015 -16. Pp. 11 Queensland Government, Brisbane.

QUEENSLAND GOVERNMENT (2018). Ross Lobegeiger report to farmers. Aquaculture production summary for Queensland 2017-18. Pp. 8 Queensland Government, Brisbane.

QUEENSLAND GOVERNMENT (2020). Ross Lobegeiger report to farmers. Aquaculture production summary for Queensland 2018-19. Pp. 8 Queensland Government, Brisbane.

RANJAN R AND BAVITHA M (2015). Lupins-An alternative protein source for aquaculture diets. *IJAR* 1(3):04-08.

RODGERS LJ, SAOUD PI AND ROUSE DB (2006). The effects of monosex culture and stocking density on survival, growth and yield of redclaw crayfish (*Cherax quadricarinatus*) in earthen ponds. *Aquaculture* 259(1):164-168. doi: https://doi.org/10.1016/j.aquaculture.2005.11.056.

RODRÍGUEZ-CANTO A, ARREDONDO-FIGUEROA JL, PONCE-PALAFOX JT AND ROUSE DB (2002). Growth Characteristics of the Australian Redclaw Crayfish, *Cherax quadricarinatus*, Cultured in an Indoor Recirculating System. *Journal of Applied Aquaculture* 12(3):59-64. doi: 10.1300/J028v12n03_06.

RODRÍGUEZ-GONZÁLEZ H, GARCÍA-ULLOA M, HERNÁNDEZ-LLAMAS A AND VILLARREAL H (2006). Effect of dietary protein level on spawning and egg quality of redclaw crayfish *Cherax quadricarinatus*. *Aquaculture* 257(1–4):412-419. doi: http://dx.doi.org/10.1016/j.aquaculture.2006.01.020.

RODRIGUEZ-GONZALEZ H, VILLARREAL H, GARCIA-ULLOA M AND HERNANDEZ-LLAMAS A (2009a). Evaluation of practical diets containing different protein levels on gonad development of female redclaw crayfish *Cherax quadricarinatus*. *Aquaculture Nutrition* 15(4):347-355. doi: 10.1111/j.1365-2095.2008.00599.x.

RODRÍGUEZ-GONZÁLEZ H, VILLARREAL H, HERNÁNDEZ-LLAMAS A, GARCÍA-ULLOA M, VÁZQUEZ-BOUCARD C AND SERRANO-PINTO V (2011). Effect of dietary protein-to-energy ratio on reproduction in female redclaw crayfish *Cherax quadricarinatus*. *Aquaculture Research* 42(12):1840-1847. doi: 10.1111/j.1365-2109.2010.02787.x.

RODRIGUEZ-GONZALEZ H, VILLARREAL H, GARCIA-ULLOA M AND HERNANDEZ-LLAMAS A (2009b). Dietary lipid requirements for optimal egg quality of redclaw crayfish, *Cherax quadricarinatus*. *Journal of the World Aquaculture Society* 40(4):531-539. doi: https://doi.org/10.1111/j.1749-7345.2009.00267.x.

ROMERO X (2002). Ups and downs of red claw crayfish farming in Ecuador. *World Aquaculture* 33(2):40-46. doi: not available.

ROMERO X AND JIMENEZ R (2002). Histopathological survey of diseases and pathogens present in redclaw crayfish, *Cherax quadricarinatus* (Von Martens), cultured in Ecuador. *Journal of Fish Diseases* 25(11):653-667. doi: 10.1046/j.1365-2761.2002.00411.x.

ROMERO XM (1998). Alter the bubble burst: redclaw farming in Ecuador. *Crayfish News (Newsletter of the International Association of Astacology)* 20(3):7-8. doi: not available.

ROUSE DB (1994). A new species for Ecuador? *World Aquaculture* 25(3):51. doi: not available.

ROUSE DB AND YEH HS (1995). Factors influencing spawning of *Cherax quadricarinatus* in indoor hatcheries. *Freshwater Crayfish* 10:605-610. doi: not available.

RUSCOE IM, JONES CM, JONES PL AND CALEY P (2005). The effects of various binders and moisture content on pellet stability of research diets for freshwater crayfish. *Aquaculture Nutrition* 11(2):87-93. doi: 10.1111/j.1365-2095.2004.00324.x.

RUSCOE IM, JONES PL AND JONES CM (2000). Effects of feeding moist and dry diets to redclaw crayfish, *Cherax quadricarinatus*, in tanks. *Freshw Crayfish* 31:164-176. doi: not available.

SALAME M (1995). Successful culture of red claw crawfish, *Cherax quadricarinatus*. Pp. 204. World Aquaculture Society.

SAMMY N (1988). Breeding biology of *Cherax quadricarinatus* in the Northern *Territory*. Pp. 79-88 *In:* Evans LH and O'Sullivan D, eds.). Proceedings of the First Australian Aquaculture Conference, Perth, 23-27 October, 1988. Curtin University of Technology, Western Australia.

SÁNCHEZ-PAZ A, GARCÍA-CARREÑO F, MUHLIA-ALMAZÁN A, PEREGRINO-URIARTE AB, HERNÁNDEZ-LÓPEZ J AND YEPIZ-PLASCENCIA G (2006). Usage of energy reserves in crustaceans during starvation: status and future directions. *Insect Biochemistry and Molecular Biology* 36(4):241-249. doi: *https://doi.org/10.1016/j.ibmb.2006.01.002*.

SAOUD IP, GARZA DE YTA A AND GHANAWI J (2012). A review of nutritional biology and dietary requirements of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868). *Aquaculture Nutrition* 18(4):349-368. doi: https://doi.org/10.1111/j.1365-2095.2011.00925.x.

SAOUD IP, GHANAWI J, THOMPSON KR AND WEBSTER CD (2013). A review of the culture and diseases of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868*). Journal of the World Aquaculture Society* 44(1):1-29. doi: *https://doi.org/10.1111/jwas.12011*.

SAOUD IP, RODGERS LJ, DAVIS DA AND ROUSE DB (2008). Replacement of fish meal with poultry by-product meal in practical diets for redclaw crayfish (*Cherax quadricarinatus*). *Aquaculture Nutrition* 14(2):139-142. doi: 10.1111/j.1365-2095.2007.00513.x.

SAVAGE J AND HOBSBAWN P (2015). Australian fisheries and aquaculture statistics 2014. FRDC project 2014/245. Australian Government, Canberra.

SEDGWICK RW (1979). Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *Penaeus merguiensis* de Man. *Aquaculture* 16(1):7-30. doi: https://doi.org/10.1016/0044-8486(79)90168-6.

SMITH TIJ AND SANDIFER PA (1979). Observations on the behavior of the Malaysian prawn, *Macrobrachium rosenbergii* (de Man), to artificial habitats. *Marine and Freshwater Behaviour and Physiology* 6(2):131-146.

doi: https://doi.org/10.1080/10236247909378560.

STEVENSON J, JERRY D AND OWENS L (2013). Redclaw Selective Breeding Project. RIRDC Publication No. 13/007 RIRDC Project No. PRJ-000327 Final Report. Pp. 47. Rural Industries Research and Development Corporation, Canberra.

STUMPF L, TROPEA C AND LÓPEZ GRECO LS (2014). Recovery growth of *Cherax quadricarinatus* juveniles fed on two high-protein diets: Effect of daily feeding following a cyclic feeding period on growth, biochemical composition and activity of digestive enzymes. *Aquaculture* 433:404-410. doi: 10.1016/j.aquaculture.2014.06.037.

TCHERKASHINA NY (1977). Survival, growth and feeding dynamics of juvenile crayfish (*Astacus leptodactylus cubanicus*) in ponds and the River Don. *Freshwater Crayfish* 3:95-100. doi: not available.

THOMPSON KR, BAILEY TJ, METTS LS, BRADY YJ AND WEBSTER CD (2010). Growth response and fatty acid composition of juvenile red claw crayfish (*Cherax quadricarinatus*) fed different sources of dietary lipid. *Aquaculture Nutrition* 16(6):604-615. doi: 10.1111/j.1365-2095.2009.00697.x.

THOMPSON KR, METTS LS, MUZINIC LA, DASGUPTA S AND WEBSTER CD (2006). Effects of feeding practical diets containing different protein levels, with or without fish meal, on growth, survival, body composition and processing traits of male and female Australian red claw crayfish (*Cherax quadricarinatus*) grown in ponds. *Aquaculture Nutrition* 12(3):227-238. doi: https://doi.org/10.1111/j.1365-2095.2006.00407.x.

THOMPSON KR, MUZINIC LA, CHRISTIAN TD, WEBSTER CD, MANOMAITIS L AND ROUSE DB (2003a). Effect on Growth, Survival, and Fatty Acid Composition of Australian Red Claw Crayfish *Cherax quadricarinatus* Fed Practical Diets With and Without Supplemental Lecithin and/or Cholesterol. *Journal of the World Aquaculture Society* 34(1):1-10. doi: https://doi.org/10.1111/j.1749-7345.2003.tb00033.x.

THOMPSON KR, MUZINIC LA, CHRISTIAN TD, WEBSTER CD, MANOMAITIS L AND ROUSE DB (2003b). Lecithin requirements of juvenile Australian red claw crayfish *Cherax quadricarinatus. Aquaculture Nutrition* 9(4):223-230. doi: https://doi.org/10.1046/j.1365-2095.2003.00246.x.

THOMPSON KR, MUZINIC LA, ENGLER LS, MORTON S-R AND WEBSTER CD (2004). Effects of feeding practical diets containing various protein levels on growth, survival, body composition, and processing traits of Australian red claw crayfish (*Cherax quadricarinatus*) and on pond water quality. *Aquaculture Research* 35(7):659-668. doi: https://doi.org/10.1111/j.1365-2109.2004.01063.x.

THOMPSON KR, MUZINIC LA, ENGLER LS AND WEBSTER CD (2005). Evaluation of practical diets containing different protein levels, with or without fish meal, for juvenile Australian red claw crayfish (*Cherax quadricarinatus*). *Aquaculture* 244(1-4):241-249. doi: https://doi.org/10.1016/j.aquaculture.2004.11.018.

TIDWELL JH, COYLE S, WEBEL C AND EVANS J (1999). Effects and interactions of stocking density and added substrate on production and population structure of freshwater prawns *Macrobrachium rosenbergii*. *Journal of the World Aquaculture Society* 30(2):174-179. doi: https://doi.org/10.1111/j.1749-7345.1999.tb00864.x.

TLUSTY MF AND LAGUEUX K (2009). Isolines as a new tool to assess the energy costs of the production and distribution of multiple sources of seafood. *Journal of Cleaner Production* 17(3):408-415. doi: https://doi.org/10.1016/j.jclepro.2008.08.001.

VAZQUEZ FJ, CHAULET A, BUGNOT A AND LOPEZ-GRECO LS (2004). Effect of temperature on sexual differentiation in juveniles of the freshwater crayfish, *Cherax quadricarinatus* (Astacida, Parastacidae). Pp. 255-259. Proceedings of the III Gongreso Iberoamerican Virtual de Acuiculura.

VIAU VE, OSTERA JM, TOLIVIA A, BALLESTER ELC, ABREU PC AND RODRÍGUEZ EM (2012). Contribution of biofilm to water quality, survival and growth of juveniles of the freshwater crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae). *Aquaculture* 324-325:70-78. doi: 10.1016/j.aquaculture.2011.10.009.

VIAU VE AND RODRÍGUEZ EM (2009). Substrate selection and effect of different substrates on survival and growth of juveniles of the freshwater crayfish *Cherax quadricarinatus* (von Martens 1868) (Decapoda, Parastacidae). *Aquaculture International* 18(5):717-724. doi: 10.1007/s10499-009-9292-0.

VOLPE MG, SANTAGATA G, COCCIA E, DI STASIO M, MALINCONICO M AND PAOLUCCI M (2015). Pectin-based pellets for crayfish aquaculture: structural and functional characteristics and effects on Redclaw *Cherax quadricarinatus* performances. *Aquaculture Nutrition* 21(6):814-823. doi: https://doi.org/10.1111/anu.12204.

WEBSTER CD, THOMPSON KR, MUZINIC LA, YANCEY DH, DASGUPTA S, XIONG YL, ROUSE DB AND MANOMAITIS L (2004). A preliminary assessment of growth, survival, yield, and economic return of Australian red claw crayfish, *Cherax quadricarinatus*, stocked at three densities in earthen ponds in a cool, temperate

climate. *Journal of Applied Aquaculture* 15(3-4):37-50. doi: https://doi.org/10.1300/J028v15n03_03.

WOUTERS R, LAVENS P, NIETO J AND SORGELOOS P (2001). Penaeid shrimp broodstock nutrition: an updated review on research and development. *Aquaculture* 202(1-2):1-21. doi: https://doi.org/10.1016/S0044-8486(01)00570-1.

XUE XM, ANDERSON AJ, RICHARDSON NA, XUE GP AND MATHER PB (1999). Characterisation of cellulase activity in the digestive system of the redclaw crayfish (*Cherax quadricarinatus*). *Aquaculture* 180(1999):373-386. doi: https://doi.org/10.1016/S0044-8486(99)00213-6.

YEH HS AND ROUSE DB (1994). Indoor spawning and egg development of the red claw crayfish *Cherax quadricarinatus*. *Journal of the World Aquaculture Society* 25(2):297-302. doi: https://doi.org/10.1111/j.1749-7345.1994.tb00194.x.

ZENTENO-SAVÍN T, CORTES-JACINTO E, VÁZQUEZ-MEDINA JP AND VILLARREAL-COLMENARES H (2008). Oxidative damage in tissues of juvenile crayfish (*Cherax quadricarinatus* von Martens, 1868) fed with different levels of proteins and lipid. . *Hidrobiológica* 18(2):147-154. doi: not available.

ZHAO Y, MENG F, CHEN L, GU Z, XU G AND LIU Q (2000). Effects of Different Gradient Temperatures on Embryonic Development of the *Cherax quadricarinatus* (Crustacea, Decapoda). *Journal of Lake Sciences* 12(1):59-62. doi: not available.

ZHU H, JIANG Q, WANG Q, YANG J, DONG S AND YANG J (2013). Effect of dietary carbohydrate-to-lipid ratios on growth performance, body composition, hepatic enzyme activities, and digestive enzyme activities of juvenile Australian Redclaw Crayfish, *Cherax quadricarinatus* (von Martens). *Journal of the World Aquaculture Society* 44(2):173-186. doi: https://doi.org/10.1111/jwas.12024.

Chapter 2: Evaluation of Four Practical Diets on the Growth and Survival of Juvenile Redclaw, *Cherax quadricarinatus* (von Martens, 1868)

A Version of this Chapter has been published as:

Rigg DP, Courtney RL, Seymour JE and Jones CM (2021). Evaluation of Four Practical Diets on the Growth and Survival of Juvenile Redclaw, *Cherax quadricarinatus* (von Martens, 1868). Freshwater Crayfish 26(1): 1-8. doi**:** Not Yet Available.

Statement of the Contribution of Others

Damian Rigg is the primary author of this chapter and was extensively involved in all aspects of this work under the supervision of: Associate Professor Clive Jones, Professor Jamie Seymour and Dr. Robert Courtney. Jessica Sleeman and Sally Browning assisted with animal husbandry, numerous aquarium volunteers also assisted with animal husbandry. Simon Irvin of CSIRO Bribie Island Research Centre (BIRC) supplied experimental diets. Financial support was supplied by AgriFutures Australia (formerly RIRDC) which provided all necessary equipment and funding for travel and conference attendance. The research was supported by the North Queensland Crayfish Farmer's Association and AquaVerde Redclaw Crayfish Farm and Hatchery which supplied experimental animals and broodstock (www.AquaVerde.com.au), grateful thanks to Colin and Ursula Valverde. The Australian Postgraduate Award (APA) scholarship provided financial support for Damian Rigg during this candidature. We thank the Freshwater Crayfish editor and the two anonymous reviewers for their useful comments and suggestions.

Chapter 2: Evaluation of Four Practical Diets on the Growth and Survival of Juvenile Redclaw, *Cherax quadricarinatus* (von Martens, 1868)

2.1 Abstract

Redclaw have shown promise as an aquaculture species, however commercial development has been constrained by variability of production. This may be a result of poor survival and growth during early juvenile stages. Diet is a critical factor that contributes to survival and growth, and although previous studies have determined nutrient and feeding requirements for on-growing larger juvenile and adult redclaw, little research has been applied to the initial juvenile stages after hatching. An experiment was performed to evaluate survival and growth of early instar redclaw using four different diets; Frippak (commercially-available post-larval shrimp diet), a compound diet formulated by CSIRO, bloodworms and on-grown *Artemia*. Bloodworms and *Artemia* produced significantly higher survival of juveniles than those fed the other diets. Juveniles fed either *Artemia* or Frippak had significantly higher weight increase over the two-week period, compared with bloodworms or the CSIRO diet. Biomass of redclaw juveniles was significantly higher when fed *Artemia*. High mortality in the Frippak and CSIRO diet treatments occurred between days six and nine of the experiment. Mortalities were not wholly attributable to nutritional deficiencies as the manufactured diets became bound and less accessible which may have reduced intake, compromising the crayfish and leading to difficulties completing ecdysis and eventual death. This study concluded that *Artemia* and bloodworms promoted highest survival, and *Artemia* and Frippak the highest weight gain. The best combination of survival, weight gain and biomass amongst the four diets trialed was with the *Artemia* diet.

2.2 Introduction

Redclaw, *Cherax quadricarinatus* (Parastacidae), is a large and commercially attractive freshwater crayfish aquaculture species, endemic to the Gulf of Carpentaria catchment of northern Australia, and southern Papua New Guinea (Jones 1990b; Jones and Ruscoe 2002; Webster *et al*. 2004; Bugnot and Lopez Greco 2009; Ghanawi and Saoud 2012; Saoud *et al*. 2013; Zhu *et al*. 2013; Stumpf *et al*. 2014). The genus *Cherax* includes several species endemic to and of commercial interest in Australia including *Cherax destructor* (Clark) and *Cherax albidis* (Clark), collectively referred to as yabbies, *Cherax tenuimanus* (Smith) and *Cherax cainii* (Austin), collectively referred to as marron, and *C. quadricarinatus* referred to as redclaw (Masser and Rouse 1997). Although long known to locals of the remote areas where it is endemic and highly valued as a food resource, redclaw was first introduced to the broader public in the late 1980's in southeast Queensland as an exciting new aquaculture species (Jones 1990b).

From the initial attempts to farm this species through to the present, redclaw has shown potential and great promise to be a significant aquaculture candidate (Jones 1990a). It is considered a generally robust species with beneficial physical, biological and commercial properties that translate well to farming in sub-tropical and tropical areas worldwide (Jones and Ruscoe 1996). Its physical robustness, comparatively simple life cycle and production technology, combined with low protein food requirement (Jones and Ruscoe 1996), suggest it is economically viable to produce (FAO 2017).

Despite these positive credentials, commercial aquaculture production of redclaw in Australia peaked in 2005/06 at 105 t with an average of only 66 t per annum since 1995 (Queensland Government 2016). In 2017/18 production was only 48.8 t (Queensland Government 2018). Part of the reason for the relatively low level of

production and lack of industry growth is the high variability of production from grow-out operations, which has constrained new investment. Such production variability has been attributed to high mortality of the juveniles when first stocked to grow-out ponds due to their small size and vulnerability to predation. Exacerbating this, there is a lack of knowledge about the specific requirements for the earliest juvenile stages in regard to diet, temperature, habitat and stocking density. Variability in survival and growth rates in the grow-out of redclaw may also be due to the extensive / semi-intensive nature of production practices used in the redclaw farming industry, in contrast with more intensive, managed approaches of well-established aquaculture industries such as shrimp and finfish aquaculture. Intensification has been a key factor in reducing variability of survival and growth rate of commercial aquaculture species, leading to enhanced stability, investment and growth of the industry. Redclaw aquaculture will likely benefit from such intensification of farming practices, and the recent development of a redclaw hatchery system to mass-produce crayling seedstock for grow-out is a positive sign (Jones *et al*. 2018; Jones and Valverde 2020).

Current redclaw aquaculture industry practice is to purchase the hatchery produced crayling stage (2 moults post egg hatch) (Parnes and Sagi 2002) and stock them directly into earthen ponds for on-growing for approximately six to nine months to reach marketable size (Stevenson 2013). This results in high variability of production, with survival ranging from <10% to greater than 70%. Inconsistency is the greatest threat to the farmer and industry consensus is that the crayling stage may be too small and vulnerable for direct pond stocking.

The crayling is the first independent, post-larval stage when exogenous feeding begins. Typical grow-out ponds of 1,000 to 2,000 m^2, are stocked at a density of 10 per m^2 (Jones 1995b, c, d). Each crayling is around 0.02 g in size, therefore a 1000 m^2 grow-

out pond would be stocked with 200 g of craylings. This relatively small biomass in such a large area is an inefficient use of farm resources and subject to the vagaries of the environment. This has highlighted the lack of knowledge about the requirements of craylings in terms of diet, temperature, habitat/shelter and predation immediately after their release into grow-out ponds (Manor *et al*. 2002). The study reported here examined the effect of different diets on crayling stage redclaw with a view toward defining optimal feeding practices and developing specific nursery protocols to enable high survival and acceptable growth rates through to a more advanced juvenile stage.

The study assessed the effect of four diets; Frippak PL + 300 Ultra (a commercially-available post-larval shrimp diet), a compound diet with formulation and production by CSIRO (Commonwealth Scientific and Industrial Research Organisation) (RC-16-2) and two natural feeds, frozen bloodworms (Chironomidae) and frozen on-grown *Artemia*.

2.3 Materials and Methods

2.3.1 Craylings

A breeding facility, consisting of four 2000 L tanks, was stocked with nine mature male and 16 mature female redclaw, each provided with individual 90 mm diameter PVC tubes of c. 200 mm length for shelter/habitat. To stimulate the redclaw to reproduce outside the natural spring/summer breeding period from September to April, the thermal and photoperiod regimes were managed to enable the anticipated need to supply craylings for experiments after April and on-going throughout the year. Water temperature was stabilized at 27°C +/- 1°C from ambient water temperature (air temperature daily average 23 °C – 30°C) using a heat pump, and the photoperiod was set to 14L:10D, using timer adjustable lighting as per methods applied for *C. quadricarinatus* by Parnes and Sagi (2002). This protocol was successful in producing

berried females, and one female redclaw crayfish (OCL = 49 mm, 76.10 g) with post-larval, stage 2 juveniles attached (Levi *et al*. 1999; Garcia-Guerrero *et al*. 2003), was chosen for the study. The female with attached stage 2 juveniles was moved to the experimental laboratory, placed in a 300 mm x 300 mm x 300 mm aquarium and held for five days until the juveniles had completed a further moult to stage 3 craylings , and began making forays away from the maternal pleopods. These craylings were examined to confirm they were all stage 3 juveniles and were carefully isolated. Excess water was removed from the craylings by placing them on absorbent paper for three seconds and each crayling was weighed to the nearest tenth of a milligram prior to being placed in the individual experimental baskets. At the conclusion of the experiment this weighing procedure was repeated. The diet experiment was performed over two weeks to allow for at least one moult. Offspring from a single female were used in order to avoid the possible confounding effects of multiple genetic sources (Austin 1986; Jones 1995a).

2.3.2 Diet Treatments

The shrimp aquaculture industry commonly uses commercial, manufactured feeds for post-larval stages, and these may be suited to early stage juvenile redclaw. The commercial diet Frippak PL+ 300 (specified for shrimp stages PL4 to PL8), was chosen as one of the diet treatments as it had been previously used successfully for early instar redclaw (Meade and Watts 1995). An experimental compound diet formulated by CSIRO (RC-16-2) was the second diet chosen for the experiment. Its formulation was based on a review of redclaw nutrition literature and designed specifically for early stage juvenile redclaw (Pavasovic 2008; Kobayashi *et al*. 2015; Pirozzi 2016), and prepared as an extruded diet in crumble form (300 to 500 µm). For the third and fourth diet treatments, two whole organism foods were chosen on basis that such food had proved successful in other redclaw studies (Jones 1995b; Parnes and Sagi 2002). These

two diets consisted of on-grown *Artemia* and whole Chironomid worms. The *Artemia* were frozen and supplied from Aqua One, and bloodworms (Chironomidae) supplied from Aquarium Industries, in Australia. Both feeds are commonly used by redclaw farmers to feed craylings (North Queensland Redclaw Farmer's Association, personal communication).

The four diets were therefore, Frippak PL +300, CSIRO manufactured diet, *Artemia* and bloodworms. The proximate composition of the diets are presented in Table 1.

Table 1. Proximate composition of diets used for juvenile redclaw. Data as supplied by the manufacturer.

Diet	Crude protein (% dry weight)	Crude lipid (% dry weight)
Frippak PL +300	51.9%	8.6%
CSIRO Diet	37.1%	4.9%
Artemia	57.0%	8.4%
Bloodworms	90.0%	4.0%

2.3.3 Replicated Experimental Recirculating Systems

Each of the four diet treatments were assigned to one of four replicated recirculating aquarium systems, each system consisting of five tanks (8 L) with a flow rate of 120 L H^{-1} (three exchanges per hour) and one sump (10 L) with a total system water volume of 50 L. Of the five tanks in each system only three were used for the experiment (i.e. three replicates). Water temperature was controlled using 50 W in-line heaters to achieve a constant 26ºC +/- 1ºC consistent with existing industry protocols. The fourth and fifth tanks of each system had no experimental baskets but did hold system water, to increase system volume and buffering capacity. Mechanical filtration was via a filter sock (pore size 200 µm, 130 x 180 mm) fitted over the sump inlet, which

was removed and cleaned daily. Biological filtration was achieved via a mixture of 20 mm and 30 mm bio-balls that were preconditioned prior to the experiment with nitrogen-fixing bacteria. These bio-balls were held in a plastic drainage basket beneath the filter sock. Three of the experimental tanks in each experimental system contained a stainless-steel rack holding 30 slotted plastic baskets of 50 ml capacity (50 x 35 mm top dimension, depth = 60 mm, 20 x 35 mm bottom dimension). Each basket was modified by the addition of sealant to the bottom 10 mm to prevent food loss. Each treatment had a total of 90 craylings which were each held individually in baskets. The water was delivered to each tank via a spray bar under the water surface, which was designed to push water across the tanks and through the baskets to assist in the flushing of waste and excess food, and to increase water and oxygen exchange within the baskets. A weighted foam pad covered all 30 baskets to prevent craylings moving between baskets, prevent cannibalism, and to allow for the tracking of individuals over the course of the experiment. The crayfish were kept in darkness by keeping an opaque lid on each tank. The lids were lifted daily for cleaning (each rack lifted and swirled to remove remaining food items from the previous day) and feeding. The craylings were fed according to the following protocol and mortalities were counted daily.

Each of the feed types required different preparation protocols. The frozen bloodworms were defrosted and mixed with dechlorinated tap water at the ratio of 10 g water: 1 g Bloodworms. The frozen *Artemia* were defrosted and mixed with dechlorinated tap water at the ratio of 10 g water: 1 g *Artemia*. The Frippak diet was mixed with dechlorinated tap water at the ratio of 10 g water: 1 g Frippak. The CSIRO diet was presented as a crumble of 300 to 500 μm size. The crumble was mixed with dechlorinated tap water at the ratio of 10 g water: 1 g CSIRO diet. This step was applied to reduce potential physical handling effects as reported by Meade and Watts (1995).

Each crayling was fed once per day via a 6 ml pipette at the quantity of 1 droplet (~ 0.03 ml) per individual animal. The prepared feed was placed directly into the bottom of each experimental basket, which allowed for each crayling to feed to satiation daily.

2.3.4 Water Quality

Tap water which had been heavily aerated for 48 hours to remove free chlorine was used to initially fill the systems, and to perform water exchanges of 34 L per system per day (~ 70%). Ammonia and nitrite readings were taken daily throughout the experiment using an API Freshwater Master Test Kit. Temperature, pH and dissolved oxygen readings were taken on days 2 and 9 with a YSI Professional Plus water quality meter (Yellow Springs Instrument Company Ohio USA).

2.3.5 Statistical Analyses

The mean weight of the different diet treatment portions fed to individual craylings were recorded and the data was analysed via a One-Way Analysis of variance for the independent variable diet treatment weight, with *post hoc* least significant difference (LSD) tests (diet treatment weight = dependent variable, diet [4 levels] = independent variable).

Weight gain was calculated as a percentage of the starting weight for each crayling in each diet treatment, arcsine√ transformed and analysed for the independent variable, diet treatment, via a One-Way Analysis of Variance, with *post hoc* LSD tests (percent weight gain = dependent variable, diet [4 levels] = independent variable). These data were similarly analysed for the independent variable tank [3 levels].

The daily crayling mortality data were analysed via a Kaplan-Meier survivorship curve with a comparison of survival functions for diet treatment, analysed via a log rank (Mantel-Cox) Chi –Square analysis, as per Jelkić *et al.* (2014). Survival time was defined as the elapsed number of days from the beginning of the experiment.

The survival of *C. quadricarinatus* craylings data was analysed as a percentage of the starting density, arcsine√ transformed, and survival of craylings over the two week trial period was compared via a One-Way Analysis of Variance, with *post hoc* LSD tests (percent survival = dependent variable, diet [4 levels] = independent variable). These data were similarly analysed for the independent variable tank [3 levels].

To quantify the starting biomass of the craylings, the mean weight of craylings in each tank (n = 3) per diet treatment was compared via a One-Way Analysis of variance with *post hoc* LSD tests (crayling weight = dependent variable, diet [4 levels] = independent variable). Biomass at the completion of the experiment was similarly analysed. Statistical analyses were conducted using IBM SPSS Statistics Version 24.

2.4 Results

Water quality parameters over the course of the experiment were; Ammonia (NH_3) ≤ 0.25 ppm, Nitrite (NO_2) = 0, Temperature 25ºC to 26.2ºC, pH $\bar{x}$ = 8.74.

The mean weight of the food for each diet delivered to individual crayfish varied between diet types. The mean weights for bloodworms and *Artemia* were not significantly different from each other (*Artemia* $\bar{x}$ = 0.0477 g, *SD* = 0.0058 g, *n* =10; bloodworms $\bar{x}$ = 0.0532 g, *SD* = 0.0096 g, *n* =10) although they were significantly greater than the Frippak and CSIRO diets (Frippak $\bar{x}$ = 0.0329 g, *SD* = 0.0054g, *n* =10, CSIRO $\bar{x}$ = 0.0338 g, *SD* = 0.0083 g, *n* =10), which were not different from one another ($F_{3, 39}$ = 18.384, $p < 0.001$). However, for all food types, there was always food found in the individual baskets of all animals when they were cleaned, indicating the crayfish were fed beyond satiation.

Craylings had a significantly higher survival rate when fed *Artemia* (97%), compared to the lower survival of craylings fed the CSIRO diet (79%), and lower again

when fed Frippak diet (4%); bloodworms (84%) had a significantly higher survival percentage than Frippak, but not significantly different from *Artemia* or the CSIRO formulation ($F_{3, 11} = 80.308$, $p < 0.001$) (Figure 4). Mortality in three of the food treatments was low (less than 2% per day). However, there was significant mortality (> 50%) for Frippak-fed craylings on day 8 (Figure 5). (Log-Rank [Mantel-Cox] $\chi^2_{3, n = 4353} = 748.396$, $p < 0.001$) (Figure 5).

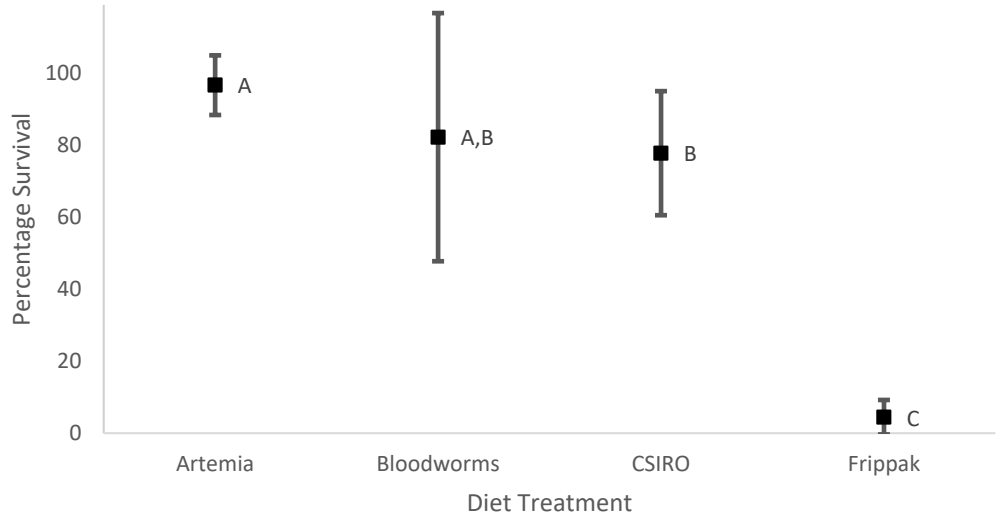

Figure 4. Average survival of Cherax quadricarinatus craylings fed four different diet types, over a two-week period.
Values presented as means, error bars represent 95% Confidence Limits. Treatment names with the same letter are not significantly different.

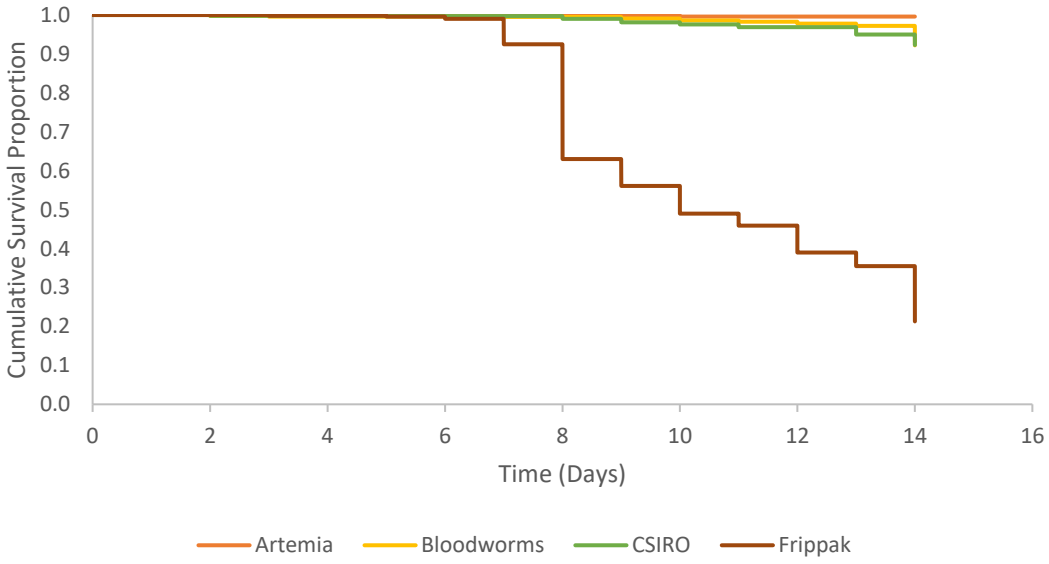

Figure 5. Kaplan-Meier survival function curves for *Cherax quadricarinatus* craylings reared with different diets over a two-week period.
Y-Axis denotes the cumulative proportion of animals surviving at the time.

Craylings had a significantly higher weight gain when fed *Artemia* (77%) than when fed bloodworms (53%) or the CSIRO diet (58%). Craylings fed the Frippak diet were not significantly different to any of the other diets (58%) ($F_{3, 230} = 24.399$, $p < 0.001$) (Figure 6).

Figure 6. Average weight change of *Cherax quadricarinatus* craylings fed different diets, over a two-week period.
Values presented as means, error bars represent 95% Confidence Limits. Treatment names with the same letter are not significantly different.

There was no significant effect of tank on the survivorship of craylings at the end of the experiment, however there was a statistically significant effect of tank on percentage weight gain $F_{2, 231}$= 4.579, p = 0.011, whereby one individual tank was significantly higher overall than the other tanks, which did not differ.

There was no statistically significant difference in the mean biomass ($\bar{x}$ = 0.4366 g, *SD* = 0.0111 g, n = 12) for craylings assigned to each of the four diet treatments at the start of the experiment ($F_{3, 11}$ = 0.392, p = 0.762). At the conclusion of the experiment, craylings fed *Artemia* ($\bar{x}$ = 0.7495 g, *SD* = 0.0370 g, n= 3) had a significantly higher biomass than craylings fed bloodworms ($\bar{x}$ = 0.5490 g, *SD* = 0.0953 g, n = 3) and the CSIRO diet ($\bar{x}$ = 0.5382 g, *SD* = 0.0467 g, n = 3), which were not significantly different from each other. The biomass for Frippak-fed craylings ($\bar{x}$ = 0.0236 g, *SD* = 0.0120 g, n = 3) was significantly lower than the other three diet treatments, ($F_{3, 11}$ = 91.226, p < 0.001) (Figure 7).

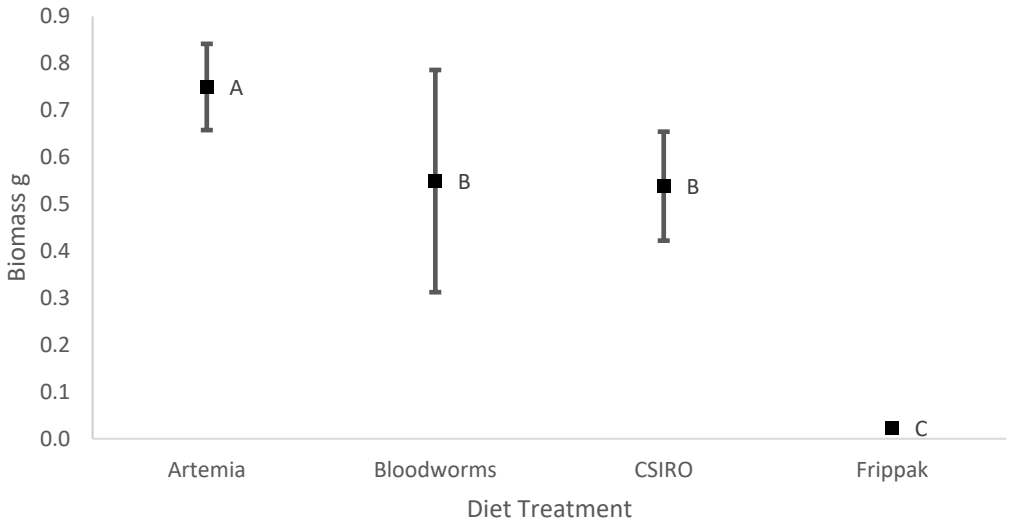

Figure 6. Mean biomass of Cherax quadricarinatus craylings fed different diet types, at the end of a two-week period.

Values presented as means, error bars represent 95% Confidence Limits. Treatment names with the same letter are not significantly different.

2.5 Discussion

Of the four diets trailed, the on-grown *Artemia* diet was the most favourable in terms of growth and survival, and would be a suitable candidate for a nursery phase based on its ready availability and low cost. The survival and weight gain of craylings fed on-grown *Artemia* combined to produce the greatest biomass at the end of the experiment. High biomass and a relatively consistent size of animals are common production goals for producers of many aquacultured species. In both respects, the *Artemia* diet amongst the four diets trailed shows promise for a nursey phase.

There was an observed tank effect whereby the percentage weight gain was significantly higher in one particular tank. This effect was driven predominantly by

craylings in that tank in the *Artemia* treatment where there were eight individuals which had more than doubled in mass over the course of the experiment (weight gain of 100.64% to 146.1%). There was only one other crayling in the experiment with over 100% weight gain which was a crayling in the CSIRO treatment (103.08%). The *Artemia* treatment had the highest mean percentage weight gain over the course of the experiment by tank, with an exception for one tank in the Frippak treatment system which had only a single surviving crayling.

The ability of the craylings to access the nutrients in the diet may help explain the high levels of mortality in some of the treatments. If the craylings were physically unable to ingest the diets offered, they may have struggled to absorb and assimilate the available proteins and lipids efficiently or effectively. This may be the case for the Frippak diet, which was of such a small particle size (300 μm, which is optimal for larval shrimp) that it might require a filter feeding capacity to enable ingestion. Juveniles of some freshwater crayfish may be capable of filter feeding as reported by Budd *et al*. (1978, 1979) however, such capability has not been confirmed for redclaw. The poor performance of both the Frippak and CSIRO diets may also be attributable to their tendency to settle as a compounded amalgam on the bottom of the baskets, rendering them less accessible. This amalgam appeared to form soon after feeding and may have prevented the craylings from filter feeding or otherwise ingesting. In addition, the food amalgam appeared to stick to the craylings appendages, impairing their ability to move and feed, and leaving them moribund. This was most acute for the Frippak diet and less so for the CSIRO diet.

The highest mortalities were recorded for craylings in the Frippak diet treatment, and there was a marked mortality spike between days six and nine (Figure 4). The timing of this mortality spike is of interest as it coincides with expected

moulting of the craylings to the next juvenile stage. Ecdysis is known to be a metabolically expensive and physiologically stressful process in which undernourished individuals can become moribund during the moult, referred to as 'moult-death syndrome'(Bowser and Rosemark 1981; Meade and Watts 1995; Anson and Rouse 1996; Thompson *et al*. 2003). The Frippak diet may not have fulfilled the nutritional requirements, either by nutritional deficiency or inaccessibility, and the physiological stress of ecdysis caused the mortality. Frippak diets are well known as being suitable for post-larval shrimp, but may not be for redclaw craylings. The external dentition of juvenile redclaw mouthparts is more raptorial than that of shrimp, and substantially different to that of adult redclaw. Such morphology is more suited to the capture and mastication of relatively large food particles such as zooplankton (copepods, ostracods, conchostracans etc.) which are known prey of redclaw juveniles (Jones 1995b), rather than fine, particulate diets.

The protein level in the diet is of importance to this discussion as redclaw juveniles are known to be highly carnivorous (Loya-Javellana *et al*. 1994; Jones 1995c). According to Cortés-Jacinto *et al*. (2009), juvenile redclaw (mean starting weight 1.04 +/- 0.3 g) require 31 to 34% protein, which complements the findings of Jones (1995b), which demonstrated a predilection for zooplankton. The juveniles in this experiment were smaller and at an earlier life stage than the juveniles in both those reported studies with a starting weight of $\bar{x}$ = 0.0143 to 0.0147 g. Saoud *et al*. (2012) suggested an ontogenetic dietary shift in redclaw, whereby juveniles have a much higher protein requirement than adults. This is supported by the present results in terms of survival and growth. Craylings fed the Frippak diet and CSIRO formulated diet performed poorly against the craylings fed the higher protein content whole-animal feeds (bloodworms and *Artemia*, Figures 4 to 7). This however is not conclusive as the

physical form of the manufactured diets appeared to have an adverse impact that may have masked the adequacy of the nutrient content.

The implications from the results are that a diet of on-grown *Artemia* shows promise as a food for a nursery phase in the aquaculture of juvenile redclaw. *Artemia* supported high survival, individual growth rate and overall biomass increase in comparison to the other diets trailed. Nevertheless, more research in respect of a nursery diet is warranted. Based on the physical morphology and nutritional requirements of *C. quadricarinatus*, future diet trials examining the enrichment of on-grown *Artemia* are advisable. There are many commercial products available to enrich *Artemia* and this could be an effective way to introduce additional beneficial nutrients into the juvenile redclaw diet, to improve health, resilience to disease and increased growth rate. In regard to manufactured diets for juvenile redclaw, further research is warranted to account for the observed preference for relatively large food particles that mimic the size and shape of the bloodworms and *Artemia*, in the form of a water-stable, semi-moist pellet.

The prospect of developing a nursery phase for redclaw aquaculture that takes the craylings now being produced in hatchery systems (Jones and Valverde 2020), to an advanced juvenile stage, best suited for grow-out pond stocking, is strongly justified. Whether such a nursery phase is managed in a tank system or pond environment is not clear, but in either system, knowledge of the nutrient requirements and feeding husbandry are critical to support high survival and production of robust juveniles, likely to thrive in the subsequent grow-out phase. Redclaw craylings are small, and even when on-grown to an advanced stage of 5 grams, the biomass involved in a mass production nursery system will be relatively low. Consequently, the amount of food required will also be relatively small, and conducive therefore to a high specification diet. On-grown *Artemia* is a suitable option, and should be used in further trials as a reference diet to

compare against, based on the results of this study. A high-specification compound diet however, with form and delivery appropriate to juvenile redclaw morphology and behaviour, remains a worthy subject for further investigation.

2.6 Literature Cited

ANSON KJ AND ROUSE DB (1996). Evaluation of several commercial feeds and Crustacean Reference Diet for juvenile Australian Red Claw crayfish, *Cherax quadricarinatus*. *Journal of Applied Aquaculture* 6(1):65-76. doi: 10.1300/J028v06n01_06.

AUSTIN C (1986). Genetic considerations in freshwater crayfish farming. Pp. 73-80 *In:* Owen P and Bowden J, Eds.). Aquaculture in Australia. Fish Farming for Profit. Rural Press Qld Brisbane.

BOWSER PR AND ROSEMARK R (1981). Mortalities of cultured lobsters, *Homarus*, associated with a moult death syndrome. *Aquaculture* 23(1-4):11-18.

BUDD T, LEWIS J AND TRACEY M (1978). The filter-feeding apparatus in crayfish. *Canadian Journal of Zoology* 56(4):695-707.

BUDD T, LEWIS J AND TRACEY M (1979). Filtration feeding in Orconectes propinquus and Cambarus robustus (Decapoda, Cambaridae). *Crustaceana. Supplement*: 131-134.

BUGNOT AB AND LOPEZ GRECO LS (2009). Structural alterations in the male reproductive system of the freshwater crayfish, *Cherax quadricarinatus* (Decapoda, Parastacidae). *J Invertebr Pathol* 102(2):160-166. doi: 10.1016/j.jip.2009.08.004.

CORTÉS-JACINTO E, CAMPA-CÓRDOVA ÁI, ASCENCIO F, VILLARREAL-COLMENARES H AND HOLGUÍN-PEÑA RJ (2009). The effect of protein and energy levels in diet on the antioxidant activity of juvenile redclaw *Cherax quadricarinatus* (von Martens, 1868) Efecto del nivel de proteína y energía en la actividad antioxidante de juveniles de langosta de agua dulce *Cherax quadricarinatus* (von Martens, 1868). *Hidrobiológica* 19(2):77-83.

FAO (2017). *Cherax quadricarinatus* (von Martens, 1868). Cultured species information program. *In:* [online] FAO Fisheries and Aquaculture Department [online]

GARCIA-GUERRERO M, HENDRICKX ME AND VILLARREAL H (2003). Description of the embryonic development of *Cherax quadricarinatus* (Von Martens, 1868) (Decapoda, Parastacidae), based on the staging method. Crustaceana V76 (3):269-280.

GHANAWI J AND SAOUD IP (2012). Molting, reproductive biology, and hatchery management of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868). *Aquaculture* 358-359:183-195. doi: 10.1016/j.aquaculture.2012.06.019.

JELKIĆ D, OPAČAK A, HORVAT D AND SAFNER R (2014). Common carp fry survival during salinity stress test: effect of feeding regime-short communication. *Veterinarski arhiv* 84(4):429-438.

JONES C (1990a). The biology and aquaculture potential of the tropical freshwater Crayfish *Cherax quadricarinatus*. *Queensland Department of Primary Industries Information Series* (qi-90028): I-XV, 1-109.

JONES C (1995a). Effect of temperature on growth and survival of the tropical freshwater crayfish *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae). *Freshwater Crayfish* 8:391-398.

JONES C, VALVERDE C AND RIGG D (2018). Redclaw Crayfish Hatchery. Development of Mass Production Hatchery for *Cherax quadricarinatus*. The Association of Astacology's Twenty-Second International Symposium on Freshwater Crayfish. International Association of Astacology, Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, USA.

JONES CM (1990b). Biology and aquaculture potential of the tropical Australian freshwater crayfish (*Cherax quadricarinatus*). Pp. 19. Abstracts, Eighth International Symposium of the International Association of Astacology, Baton Rouge, USA, April 22-26, 1990. International Association of Astacology, Baton Rouge.

JONES CM (1995b). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) I. Development of hatchery and nursery procedures. *Aquaculture* 138(1995):221-238.

JONES CM (1995c). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) II. Juvenile nutrition and habitat. *Aquaculture* 138(1995):239-245. doi: 10.1016/0044-8486(95)00069-0.

JONES CM (1995d). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) III. Managed pond production trials. *Aquaculture* 138(1995):247-255. doi: 10.1016/0044-8486(95)00067-4.

JONES CM AND RUSCOE I (1996). Production Technology for Redclaw Crayfish (*Cherax quadricarinatus*). Final Report FRDC Project 92/119. Fisheries Research and Development Corporation, Canberra.

JONES CM AND RUSCOE IM (2002). Biological and aquaculture characteristics of five stocks of redclaw, *Cherax quadricarinatus* (von Martens) (Decapoda: Parastacidae) from northern Queensland, Australia Freshwater Crayfish. Pp. 115-135. Proceedings of the Thirteenth Symposium of the International Association of Astacology.

JONES CM AND VALVERDE C (2020). Development of Mass Production Hatchery Technology for the Redclaw Crayfish, *Cherax quadricarinatus Freshwater Crayfish* 25(1):1-6. doi: https://doi.org/10.5869/fc.2020.v25-1.001.

KOBAYASHI Y, WEBSTER CD, THOMPSON KR, CUMMINS JR VC, GANNAM AL, TWIBELL RG, HYDE NM AND ALBERS KOCH JF (2015). Effects on growth, survival, body composition, processing traits and water quality when feeding a diet without vitamin and mineral supplements to Australian red claw crayfish

(*Cherax quadricarinatus*) grown in ponds. *Aquaculture research* 46(11):2716-2727.

LEVI T, BARKI A, HULATA G AND KARPLUS I (1999). Mother-offspring relationships in the red-claw crayfish *Cherax quadricarinatus*. Journal of Crustacean Biology 19(3):477-484.

LOYA-JAVELLANA GN, FIELDER DR AND THORNE MJ (1994). Ontogeny of foregut in the tropical freshwater crayfish, *Cherax quadricarinatus* von Martens, 1868 (Parastacidae: Decapoda). *Invertebrate reproduction & development* 25(1):49-58.

MANOR R, SEGEV R, LEIBOVITZ MP, AFLALO ED AND SAGI A (2002). Intensification of redclaw crayfish *Cherax quadricarinatus* culture: II. Growout in a separate cell system. *Aquacultural Engineering* 26(4):263-276.

MASSER MP AND ROUSE BD (1997). Australian Red Claw Crayfish. SRAC Publication No.244. The Alabama Cooperative Extension Service, USA.

MEADE ME AND WATTS SA (1995). Weight gain and survival of juvenile Australian crayfish *Cherax quadricarinatus* fed formulated feeds. *Journal of the World Aquaculture Society* 26(4):469-474.

PARNES S AND SAGI A (2002). Intensification of redclaw crayfish *Cherax quadricarinatus* culture: I. Hatchery and nursery system. *Aquacultural Engineering* 26(4):251-262.

PAVASOVIC A (2008). Evaluation of the nutritional requirements of redclaw crayfish, *Cherax quadricarinatus*. Queensland University of Technology.

PIROZZI IJ, M. CLAY, S. OWENS, L. CONRADO, D. LE, H. NGUYEN, M. PICHETTE, A. AND STRIGHT, T. (2016). Boosting redclaw industry productivity with improved nutrition and feed management. Rural Industries Research and Development Corporation, Canberra.

QUEENSLAND GOVERNMENT (2016). Ross Lobegeiger Report to Farmers. Aquaculture production summary for Queensland 2015 - 16.

QUEENSLAND GOVERNMENT (2018). Ross Lobegeiger report to farmers. Aquaculture production summary for Queensland 2017-18.

SAOUD IP, GARZA DE YTA A AND GHANAWI J (2012). A review of nutritional biology and dietary requirements of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868). *Aquaculture Nutrition* 18(4):349-368. doi: 10.1111/j.1365-2095.2011.00925.x.

SAOUD IP, GHANAWI J, THOMPSON KR AND WEBSTER CD (2013). A review of the culture and diseases of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868). *Journal of the World Aquaculture Society* 44(1):1-29.

STEVENSON J (2013). Redclaw selective breeding project *In:* Australian Government Rural Industries Research and Development Corporation, RIRDC Project No. PRJ – 0003274800, No.13/007.

STUMPF L, TROPEA C AND LÓPEZ GRECO LS (2014). Recovery growth of *Cherax quadricarinatus* juveniles fed on two high-protein diets: Effect of daily feeding following a cyclic feeding period on growth, biochemical composition and activity of digestive enzymes. *Aquaculture* 433:404-410. doi: 10.1016/j.aquaculture.2014.06.037.

THOMPSON K, MUZINIC L, CHRISTIAN T, WEBSTER C, MANOMAITIS L AND ROUSE D (2003). Lecithin requirements of juvenile Australian red claw crayfish *Cherax quadricarinatus. Aquaculture Nutrition* 9(4):223-230.

WEBSTER CD, THOMPSON KR, MUZINIC LA, YANCEY DH, DASGUPTA S, XIONG YL, ROUSE DB AND MANOMAITIS L (2004). A preliminary assessment of growth, survival, yield, and economic return of Australian red claw crayfish, *Cherax quadricarinatus*, stocked at three densities in earthen ponds in a cool, temperate climate. *Journal of Applied Aquaculture* 15(3-4):37-50.

ZHU H, JIANG Q, WANG Q, YANG J, DONG S AND YANG J (2013). Effect of dietary carbohydrate-to-lipid ratios on growth performance, body composition, hepatic enzyme activities, and digestive enzyme activities of juvenile Australian Redclaw Crayfish, *Cherax quadricarinatus* (von Martens). *Journal of the World Aquaculture Society* 44(2):173-186.

Chapter 3: Determining Suitable Thermal Regimes for Early Instar Redclaw juveniles *Cherax quadricarinatus* (von Martens, 1868) (Decapoda, Parastacidae) For a Proposed Nursery Phase.

A Version of this Chapter has been published as:

Rigg DP, Courtney RL, Seymour JE and Jones CM (2021). Determining Suitable Thermal Regimes for Early Instar Redclaw Juveniles, *Cherax quadricarinatus* (von Martens, 1868) (Decapoda, Parastacidae), for a Proposed Nursery Phase. Freshwater Crayfish 26(1): 1-7. doi: Not Yet Available.

Statement of the Contribution of Others

Damian Rigg is the primary author of this chapter and was extensively involved in all aspects of this work under the supervision of: Associate Professor Clive Jones, Professor Jamie Seymour and Dr. Robert Courtney. Jessica Sleeman and Sally Browning assisted with animal husbandry, numerous aquarium volunteers also assisted with animal husbandry. Financial support was supplied by AgriFutures Australia (formerly RIRDC) which provided all necessary equipment and funding for travel and conference attendance. The research was supported by the North Queensland Crayfish Farmer's Association and AquaVerde Redclaw Crayfish Farm and Hatchery which supplied experimental animals and broodstock (www.AquaVerde.com.au), grateful thanks to Colin and Ursula Valverde. The Australian Postgraduate Award (APA) scholarship provided financial support for Damian Rigg during this candidature. We thank the Freshwater Crayfish editor and the two anonymous reviewers for their useful comments and suggestions.

Chapter 3: Determining Suitable Thermal Regimes for Early Instar Redclaw juveniles *Cherax quadricarinatus* (von Martens, 1868) (Decapoda, Parastacidae) For a Proposed Nursery Phase.

3.1 Abstract

Modern, intensified aquaculture typically involves three production phases; hatchery, nursery and grow-out. For redclaw crayfish aquaculture however, such delineation has been ill-defined. Farming of redclaw was initiated based on the putative beneficial physical and biological attributes of the species, which suggested production methods would be relatively simple. The simple approach proved to be inefficient and only partially effective, which hindered industry development. Hatchery technology now exists to supply seed stock for grow-out, but hatchery production is variable, and the performance of hatchery reared juveniles is inconsistent. A nursery phase has been proposed between hatchery production and grow-out of approximately 3 weeks duration, sufficient to allow 2 or more moults. An important primary parameter in the proposed nursery phase is the thermal regime that will support optimum survival and growth. This study quantified the effect of temperature on the growth and survival of redclaw juveniles for a 22 day nursery phase. Temperature had a statistically significant effect on the survival of juveniles, whereby the high temperatures were associated with high mortality, and the lower temperature treatments were associated with very low mortality. Survival was 98 to 100% for craylings held at temperatures between 18°C and 22°C, and between 0% and 6% for craylings at temperature treatments of 25°C to 32°C. Mortalities within treatments between 25°C and 30°C, primarily occurred from day six to day eleven, corresponding with the initiation of moulting. Change of mass of

crayfish was significantly higher with increasing temperature between 18°C and 22°C and at individual weights that suggest they had completed a moult. This study suggests a water temperature of 22°C is optimal for survival and growth in a nursery phase.

3.2 Introduction

Cherax quadricarinatus, commonly known as redclaw, is endemic to the westerly-flowing catchments of Cape York Peninsula in Queensland, areas of the Northern Territory and southern Papua New Guinea (Jones 1990b; Jones and Ruscoe 2002; Webster *et al*. 2004; Bugnot and Lopez Greco 2009; Ghanawi and Saoud 2012; Saoud *et al*. 2013; Zhu *et al*. 2013; Stumpf *et al*. 2014). It was first trialed as an aquaculture species in the late 1980's (Jones 1990b). The potential evident in the early development of redclaw aquaculture was based on perceived advantageous physical, biological and commercial attributes, including physical robustness and market value as a premium seafood (Jones 1990a). The life cycle is simple, with larval development occurring within the egg, the species is relatively inexpensive to feed due to a low protein food requirement during grow-out and redclaw is climatically-suited to translocation to sub-tropical and tropical areas worldwide (FAO 2017).

The relative ease with which redclaw have been farmed in the past has served to impede the development of the industry, through a perceived lack of need for innovation and technical sophistication, resulting in many small, uneconomic farms that conferred a reputation of a cottage industry that applied low technology extensive practices (King 1994) with limited and sporadic supply to the market (FAO 2017). The unfulfilled potential for the industry to develop and expand has been hampered by lack of sophistication and small scale of the farming operations, and demand for the product far exceeds current supply capabilities (FAO 2017). In most advanced aquaculture industries, production is compartmentalised into specialised enterprises which focus on

discrete parts of the life cycle, comprising production of seed stock, followed by a nursery phase, and then a grow-out phase. Such specialisation ensures that high quality, advanced seed stock can be supplied to grow-out facilities optimising the business of growing the animal for market. This is especially true for species where the early life stages can be difficult to produce, such as oysters and shrimp (Paniagua-Chavez and Tiersch 2001; Arnold *et al*. 2013).

In recent years the redclaw aquaculture industry has advanced with the development of specific hatchery technology to supply seed stock to redclaw farmers who specialise in the grow-out phase (Jones and Valverde 2020). Hatchery produced seed stock are available to stock grow-out ponds at the crayling stage, which is the first independent, free-living and exogenous feeding stage for redclaw. Although this approach has many advantages, farmers report significant inconsistency in survival and low predictability of yield after the grow-out is completed, 6 to 9 months after stocking (Jones and Valverde 2020). When survival is low and yield therefore poor, it is not clear what the mortality can be attributed to. The small size and physical vulnerability of the craylings to predation is considered the most likely. To mitigate the variability, farmers have suggested a nursery phase may be necessary to on-grow the hatchery produced seed stock (craylings) to a larger, more robust advanced juvenile stage, better suited for stocking to the grow-out phase. In considering the protocols that may be applied to such a nursery phase, definition of the optimal thermal regime that supports maximum survival and growth is of central importance. Under managed nursery conditions, water temperature may be manipulated to achieve efficient and effective production of robust juveniles that in turn will contribute towards greater consistency of grow-out production and yields.

As for all poikilotherms, temperature is a fundamental factor in redclaw biology and physiology, particularly in modulation of growth (Jones 1990a; King 1994; Jones 1995c, d; Yeh and Rouse 1995; Zhao *et al*. 2000; Garcia-Guerrero *et al*. 2003; Karplus *et al*. 2003; De Bock and López Greco 2009). Little data currently exists on the suitable thermal regimes for the early life stages of redclaw.

Previously published research has reported temperature effects on redclaw egg incubation (Garcia-Guerrero *et al.* 2003), raising juvenile redclaw (King 1994; Jones 1995b; García-Guerrero *et al.* 2013), and grow-out to marketable size (Anson and Rouse 1996; Barki *et al*. 1997b; Cortés-Jacinto *et al*. 2003; Campaña-Torres *et al*. 2005, 2008; Calvo *et al*. 2011; Calvo *et al*. 2013). An optimal egg incubation temperature of 22 to 25°C was recommended for redclaw by Garcia-Guerrero *et al*. (2003) as lower temperatures resulted in a longer incubation period although with higher hatch rate of eggs. García-Guerrero *et al*. (2013) reported that juvenile redclaw (0.75 g +/- 0.23 g) raised for 90 days at various temperatures had the greatest weight increase and total biomass at 28°C, but the highest survival at 25°C (García-Guerrero *et al*. 2013). While Jones (1995b) reported consistent high survival of juvenile redclaw of around 1 g initial weight at temperatures ranging from 20°C to 28°C, and best growth at temperatures of 22°C to 28°C. A number of studies have investigated temperature effects on larger redclaw through to market size, recommending optimal water temperature of 27°C for the on-farm grow-out phase. Noting this disparity in thermal optima for different life stages, there is justification for specifically exploring the thermal requirements for early life stages, from crayling to juvenile, as these data have not been previously generated. Defining the temperature range that will support maximum growth and survival for the first three weeks from the crayling stage will contribute to the definition of optimal nursery protocols.

The aim of this project was to quantify the effect of temperature on the growth and survival of early instars from crayling through 1 to 2 moults, to juvenile stage J1 and J2, to determine suitable thermal regimes for a proposed nursery phase for redclaw aquaculture.

3.3 Materials and Methods

3.3.1 Juvenile terminology

For clarity, the nomeclature applied to the early life stages of redclaw in this study is defined as per Table 2.

Table 2. Nomeclature for early life stage of redclaw as applied in this study.

Name used	Instar	Definition	Comment
Egg		Egg up to point of hatching	A series of larval stages are completed within the egg as per Garcia-Guerrero *et al*. (2003)
L1	1	First stage after hatching	Referred to as post-embryo I by Garcia-Guerrero *et al*. (2003)
L2	2	Stage after first moult	Referred to as stage 12 post-embryo II by Garcia-Guerrero *et al*. (2003)
Crayling	3	Stage after 2nd moult	The first free-living and feeding juvenile stage
J1	4	1 moult after crayling	Juvenile
J2	5	2 moults after crayling	Juvenile

3.3.2 Experimental Recirculating Systems

Eight temperature treatments were assigned individually to eight independent recirculating systems, with separate, dedicated sumps which incorporated air stones and heater / chiller units to maintain the designated temperature treatments within a range of +/- 0.5°C. The eight systems were identical.

Each system consisted of two tanks (8 L each) with a stainless-steel rack holding 30 perforated plastic baskets of 50 ml capacity (50 x 35 mm top dimension, depth = 60

mm, 20 x 35 mm bottom dimension). Water was delivered via a spray bar in the water column to assist in water circulation and aeration of the baskets. Each basket held an individual crayling, in order to prevent cannibalism and track individual weights and mortality. There were 60 craylings per temperature treatment. Each system also had a 10 L sump, for a total system volume of 26 L, and a flow rate of 60 L H^{-1} per tank.

The baskets were topped with a foam cover and a lid to prevent crayfish escape, translocation and cannibalism. Mechanical filtration was achieved via a filter sock (pore size 200 μm, 130 x 180 mm) over the return pipe to the sump to collect waste and uneaten food.

Daily readings of ammonia and pH were measured with an API Freshwater Master Test Kit, total hardness and total alkalinity were measured weekly using Hach Aquachek 7 test strips.

3.3.3 Treatments

Water temperatures were recorded hourly for the 22-day period of the experiment via iButton temperature loggers to monitor each treatment system. These data were used to calculate mean values. Once the systems stabilised, the temperature treatment designations were confirmed as; 18°C +/- 0.5°C, 19.5°C +/- 0.5°C, 22°C +/- 0.5°C, 25°C +/- 0.5°C, 26.5°C +/- 0.5°C, 28°C +/- 0.5°C, 30°C +/- 0.5°C, 32°C +/- 0.5°C.

3.3.4 Craylings

Craylings for the experiment were hatchery-raised from eggs and supplied by AquaVerde redclaw farm in North Queensland (17°24’20”S, 145°31’32”E, AquaVerde.com.au). They were transported to the experimental laboratory facility at James Cook University in Cairns once all the crayfish had moulted from L2 to crayling. The 480 craylings used in the experiment were generated from a single broodstock

female, and were weighed individually and randomly assigned to treatment tanks. For weighing, individual craylings were placed on kitchen paper for three seconds to remove external water and then weighed on a digital balance. This procedure was applied at both the start and end of the experiment. Daily mortalities were recorded during the experiment and where possible weighed in the same fashion.

Craylings were fed frozen, on-grown adult *Artemia* (Aqua One©), which were defrosted, suspended in water and fed at a daily rate of ≥ 2 *Artemia* indivduals per crayling. As food was provided *ad libitum*, the racks were lifted and swirled in the tubs to remove uneaten feed daily. Uneaten food and waste was collected in the filter sock and removed.

3.3.5 Data Analysis

To determine the effect of temperature on survival, the percent survival was calculated for each temperature treatment after 22 days, and analyzed for the independent variable, temperature, via a One-Way Analysis of Variance. Where significant results were obtained, *post-hoc* LSD tests were conducted to identify where significant differences occurred.

To establish the effect of temperature on weight gain, the percentage increase in weight from day 1 to 22 was calculated for each temperature treatment.

$$\text{Percentage Weight Increase} = \frac{Wtd22 - Wtd1}{Wtd1} \times 100$$

Where Wt_{d1} = weight at the start of the experiment, and Wt_{d22} = weight at the end of the experiment (22 days). These data were then analyzed for the independent variable, temperature, via a One-Way Analysis of Variance. Where significant results were obtained, *post-hoc* LSD tests were conducted to identify where significant differences occurred. Treatments were excluded from *post-hoc* analysis for percentage change in original weight if there were less than 5 animals remaining alive at day 22.

Means with associated standard errors were generated for weight at the start, end and / or at the time of death for each temperature treatment, as well as the percentage weight gain and associated standard errors (Table 3). Statistical analyses were conducted using IBM SPSS Statistics Version 24.

Table 3. Mean weight at start, end and at death, and percentage weight gain over 22 days for *Cherax quadricarinatus* craylings at eight temperatures.

Treatment	Mean Wt. (g) ± SE Start	Mean Wt. (g) ± SE End	Mean Wt. (g) ± SE At death	Mean weight gain (%) ± SE
18°C	17.5 ± 0.1	19.8 ± 0.2	N/A	17.3 ± 2.0
19.5°C	17.4 ± 0.1	31.1 ± 0.5	22.3	78.0 ± 3.2
22°C	17.7 ± 0.2	38.3 ± 1.2	N/A	117.6 ± 7.3
25°C	17.3 ± 0.2	31.6 ± 2.0	28.8 ± 0.4	66.0 ± 13.6
26.5°C	17.3 ± 0.2	36.1	26.6 ± 0.4	96.2
28°C	16.9 ± 0.2	17.9 ± 0.4	24.4 ± 0.4	5.0 ± 3.0
30°C	17.4 ± 0.2	19.2	22.6 ± 0.6	19.3
32°C	17.1 ± 0.1	N/A	19.2 ± 0.6	N/A

3.4 Results

Water quality parameters over the course of the experiment were; Ammonia (NH_3) ≤ 0.25ppm, Nitrite (NO_2) = 0, pH $\bar{x}$ = 7.5, hardness <100 mg/L and alkalinity = 80 mg L^{-1}.

Temperature had a statistically significant effect on the survival of craylings, whereby high temperatures were associated with high mortality levels, and the lower temperature treatments were associated with very low mortality levels. Survival was 100%, 98% and 100% for temperature treatments 18°C, 19.5°C and 22°C. In contrast, survival was 6%, 3%, 5%, 2% and 0% at temperatures of 25°C, 26.5°C, 28°C, 30°C and 32°C ($F_{7, 15}$ = 375.991, p < 0.001) (Figure 8).

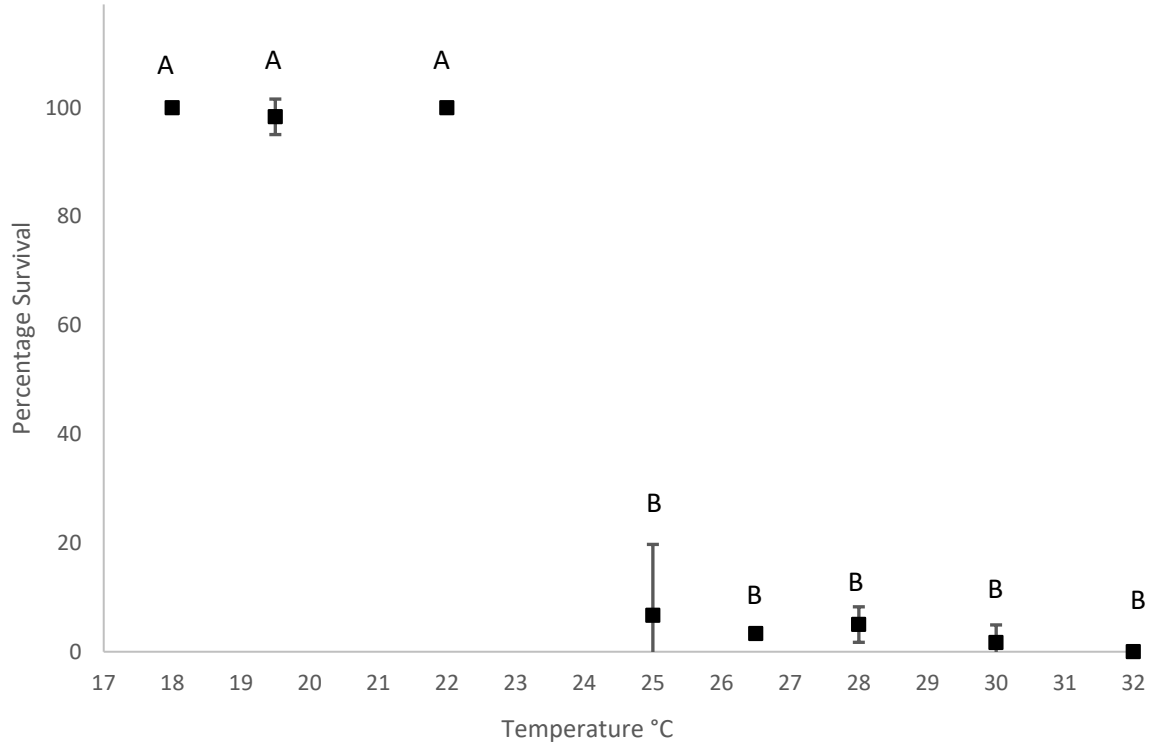

Figure 7. Percentage survival of *Cherax quadricarinatus* craylings at each of eight temperatures over the 22-day experiment.
Data points are presented as means, bars represent 95% Confidence Interval, $n = 16$. Treatments with the same letter are not significantly different.

A strong pattern emerged in the number of daily mortalities (Figure 9), with a large spike in mortalities from day six to day 11 for temperature treatments 25°C, 26.5°C, 28°C, 30°C and 32°C.

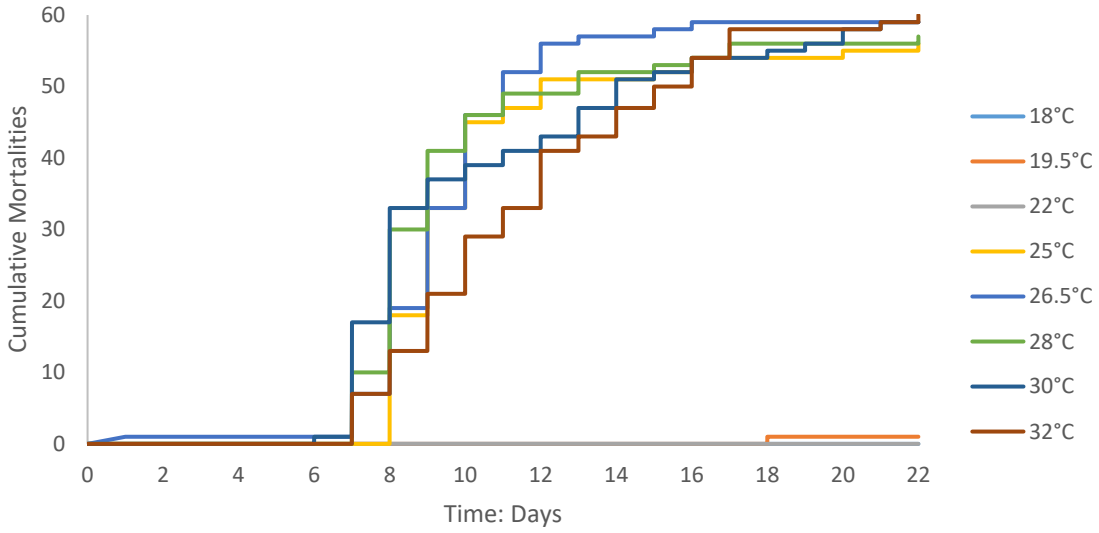

Figure 8. Cumulative crayling mortalities for *Cherax quadricarinatus* in a 22-day temperature trial.
Starting number, n = 60 per temperature treatment, cumulative mortality of 60 equals 100% mortality.

Temperature had a statistically significant effect on the change of weight of craylings. Percentage gain on original weight was significantly different between the treatments 18°C, 19.5°C and 22°C increasing with temperature up to 22°C ($F_{6, 144}$ = 20.666, p <0.001) (Figure 10). Data for weight gain for treatments 25°C, 26.5°C, 28°C, 30°C and 32°C were excluded from *post hoc* analysis due to the paucity of experimental animals remaining alive (<5).

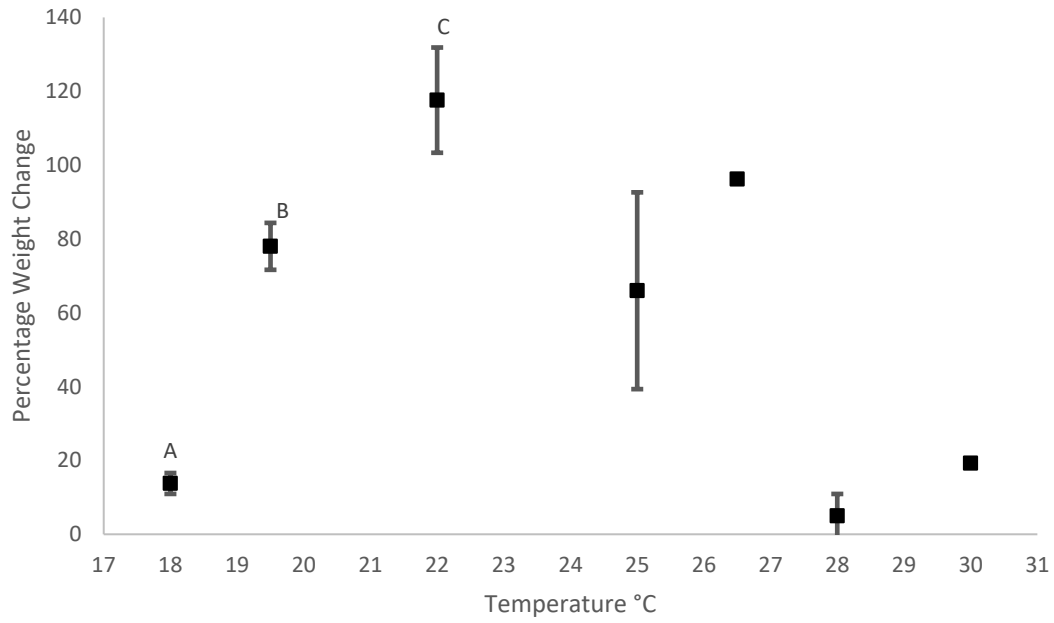

Figure 9. Weight increase of *Cherax quadricarinatus* craylings over a 22-day temperature experiment.
Mean percentage gain on original weight. Bars represent 95% confidence interval (18°C, n = 60; 19.5°C, n = 59; 22°C, n = 60; 25°C n = 4; 26.5°C, n = 1; 28°C, n = 3; 30°C, n = 1; 32°C, n = 0). Treatments with the same letter are not significantly different, treatments 25°C, 26.5°C, 28°C, 30°C and 32°C were excluded from post hoc analysis due to insufficient numbers.

3.5 Discussion

Over the course of this 22-day study, temperature was shown to have an effect on both survival and growth. There was a distinct separation in the survival of juvenile crayfish between temperatures below and above 22°C. High mortality was associated with temperatures above 22°C and very low mortality at and below 22°C (Fig. 8). The mean weight of the surviving animals from the lower temperature treatments; 18°C, 20°C and 22°C, showed a positive correlation between temperature and weight. The increase in weight of the surviving animals in all temperature treatments indicate that they had completed one moult, and in some cases two. Daily mortality exhibited a spike between day six and eleven in the treatments 25°C, 26.5°C, 28°C, 30°C and 32°C, which coincided with the expected time of moulting. Inability to complete ecdysis once

it has started is fatal to crayfish (Song *et al*. 2017) and this may explain part or all of the mortality occurring in this time period.

The process of ecdysis is extremely energy-expensive (Bowser and Rosemark 1981; Villarreal 1991) and causes severe physiological stress (Saurabh and Sahoo 2008). Inadequate nutrition can result in incomplete ecdysis where an animal becomes moribund during the moult and dies during the process, a syndrome known as exuvia entrapment disease (EED) (Saurabh and Sahoo 2008) or moult death syndrome (MDS) (Bowser and Rosemark 1981). The mortalities in treatments 25°C, 26.5°C, 28°C and 30°C, primarily occurred between day six and eleven and at a mean mortality weight (Table 3) that suggests that they had increased in size, and therefore had moulted. This indicates they died during the first moult or soon after when progressing from crayling to J1. Temperature may be a stressor or mediating factor for initiation of moult in these early stages, in that the temperature of 25°C and above stimulated onset of ecdysis more quickly than the nutritional status would allow, resulting in large numbers of mortalities. The lower temperatures would have supported lower growth rate, in effect allowing the animals to gain the nutrition required to support complete and successful ecdysis and therefore enabling one or more moults over the course of the study.

This experiment has presented evidence that the earliest free-living life stages of redclaw, from crayling to J2, may differ in temperature optima in comparison with eggs, larger juveniles and adults. Other studies have examined thermal tolerance of redclaw in slightly larger and older redclaw (0.75 g +/- 0.23 g for García-Guerrero *et al*. 2013 and 0.61 +/- 0.02 and 1.27 +/- 0.06 for Jones (1995b)), in experiments where the redclaw were stocked at a size equivalent to those at the end of the current study, i.e. more than 21 days of age (García-Guerrero *et al*. 2013). Results presented here show that the combined highest growth and survival occurred at 22°C, whereas García-

Guerrero *et al*. (2013) found highest weight gain / biomass at 28°C, highest survival at 25°C, and a thermal optimum between 23°C and 26°C. The study by (Jones 1995b) showed survival highest at temperatures of 28°C and below and best growth (i.e. above 70% of maximum) at temperature between 22°C and 31°C. A lower optimum temperature than that which produces the highest biomass or weight gain can be attributed to the stress and moult-related mortality at higher temperatures. The first free-living stage for redclaw may be more susceptible to heat stress than subsequent stages, as evidenced in this study by the high mortality at 25°C and above. Results of the current study combined with those of Jones (1995b); García-Guerrero *et al*. (2013) provide thermal data from crayling through to around 16 weeks that are consistent in regard to the deleterious effects of higher temperatures.

Lower temperatures have also been examined for redclaw using juveniles ranging in size from 0.040 to 0.046 g wet weight (King 1994) through to 0.61 +/- 0.02 g and 1.27 +/- 0.06 g (Jones 1995b), 4.35 g +/- 0.21 to 4.74 g +/- 0.24 (Prymaczok *et al*. 2012) and 36.3 +/- 1.0 g to 40.3 +/- 2.6 g and 17.3 +/- 1.0 g to 18.6 +/- 0.8 g (Karplus *et al*. 1998). The study by King (1994) used crayfish referred to as 'hatchlings' with no clear definition as to the stage of development. However, judging by the weight of the crayfish, they were probably at least J1 or J2. The survival at ten weeks for the lower temperature treatments 15°C and 20°C was 0% and 33% respectively, while 25°C and 30°C had 83% survival. The survivors in the 20°C, 25°C and 30°C treatments all grew exponentially, with a growth rate increasing with temperature up to a maximum at 30°C. These results are at odds with the results in this study, but may be accounted for by the fact that they were Mitchell River (North Queensland) wild stock from a single progeny, whereas the craylings used in this study originated from the semi-domesticated 'Walkamin' stock which may have developed different thermal

requirements. Jones (1995a) also used 'Walkamin' stock for their temperature experiments, however this was early in the domestication process of combining the Flinders and Gilbert Rivers stocks (C. Jones personnel communication), and twenty-five years later it is likely that the craylings supplied by AquaVerde redclaw farm in this instance may well have diverged genetically. Notwithstanding the genetics, the mean survival was highest at 20°C and 32°C (89%) and lowest at 34°C (23%). Mean growth in the Jones (1995a) study was highest at 28°C, followed by 24°C, 32°C, 20°C and 34°C at the end of the 70-day trial. These data largely concur with the results from our study with the notable exception that 34°C appears to be detrimental to both the survival and growth of these advanced juveniles, and the 32°C treatment showed the highest survival and only moderate growth. In another study, weight gain and survival of advanced juvenile redclaw (~5 g) were compared over 30 days at 20°C and 27°C, showing there was no significant statistical difference and that a high tolerance of lower temperatures by these larger juveniles would allow for culturing at lower temperatures with acceptable survival and growth at 20°C (Prymaczok *et al*. 2012). A study in Israel involved overwintering much sub-larger adult and adult redclaw in earthern ponds for 118 days to ascertain survival and growth where the temperature in the ponds dipped below 10°C for 6 days. Survival varied between 49% and 58.5%, however the change in weight was minimal and there was no evidence of ecdysis, suggesting the animals had not been eating (Karplus *et al*. 1998).

Considering all of the literature on temperature effects on redclaw for the successive stages from egg incubation and through the initial several instars, the optimal temperature changes. For egg incubation the optimum is 22°C to 25°C (García-Guerrero *et al*. 2013). Following this, the optimal temperature for stages L1 to L2 is currently unknown but as they are non-feeding stages development rate is likely to be

entirely reliant upon temperature. Development from crayling to J1 and J2 (22 days) as examined in the current study suggests 22°C as the optimum, while for juveniles from 3 to 16 weeks of age, optimum temperature is 23°C to 26°C (Jones 1995b; García-Guerrero *et al*. 2013). For older stages through the grow-out period greatest success appears to be at 27°C (Anson and Rouse 1996; Barki *et al*. 1997a; Cortés-Jacinto *et al*. 2003; Campaña-Torres *et al*. 2005, 2008; Calvo *et al*. 2011; Calvo *et al*. 2013). This suggests an ontogenetic or development-based shift in thermal optima as the redclaw develop from egg through to adult, progressively performing optimally at higher temperatures with increasing age and size.

This study suggests that crayling survival is high at relatively cool temperatures, for an ostensibly tropical species. Moreover, the data suggest that higher temperatures are a stressor which support faster growth but impact negatively on ecdysis. When considered in context of the natural environment however, this this is not as counter intuitive as it appears. Breeding activity in natural populations of redclaw begins at 21 to 22°C (Sammy 1988; Jones 1990c). To relate this back to the initial question which generated the study, i.e. what is the optimal thermal regime for a nursery phase, mortality was lowest for the temperatures from 18 to 22°C. The implications are that initial growth may be reduced at the lower temperatures, however if craylings experience a cooler nursery phase for a few weeks prior to release in grow-out ponds, a stronger and fitter J1 to J2 released for grow-out may potentially show enhanced survival through grow-out to market size having progressed successfully through the initial moults.

To ascertain the timing of the changes in thermal preference through the development and growth of redclaw, an experiment is required that explicitly targets the thermal tolerance of each life stage independently. A study to examine oxygen

consumption as analogous to metabolic rate should provide the required data and indicate where the change / changes in thermal preference occur in the life stages. A combination of these studies may ascertain the ideal temperature to hold each of these stages in order to provide the most advantageous temperature environment from hatchery through a nursery phase for survival and weight gain, with a view to generating the most robust juveniles, best equipped to flourish in the subsequent grow-out phase.

This study illustrates that if craylings continue to be released directly into grow-out ponds, without the addition of a nursery phase to take them through to J1 or J2, low temperature at the time of stocking should not prove to be detrimental in terms of survival, merely slowing their initial growth. Relatively high temperatures (above 22°C) may however prove to be highly detrimental to survival at these early life stages. Cooler temperatures slow development time during a critical life history change point which may lead to higher survival, lower pathogen load, lower food requirements and increased robustness. The implications are that craylings could be stocked into grow-out ponds earlier in the season when temperatures are lower to maximise grow-out time and potentially increase returns at harvest, or, after a nursery phase to support higher survival of the crayfish, that will lead to greater consistency of yields and also higher income.

3.6 Literature Cited

ANSON KJ AND ROUSE DB (1996). Evaluation of several commercial feeds and Crustacean Reference Diet for juvenile Australian Red Claw crayfish, *Cherax quadricarinatus*. *Journal of Applied Aquaculture* 6(1):65-76. doi: 10.1300/J028v06n01_06.

ARNOLD SJ, COMAN GJ AND EMERENCIANO M (2013). Constraints on seedstock production in eighth generation domesticated *Penaeus monodon* broodstock. *Aquaculture* 410:95-100. doi: 10.1016/j.aquaculture.2013.06.023

BARKI A, LEVI T, HULATA G AND KARPLUS I (1997a). Annual cycle of spawning and molting in the red-claw crayfish, *Cherax quadricarinatus*, under laboratory conditions. *Aquaculture* 157(3-4):237-247. doi: 10.1016/S0044-8486(97)00163-4

BARKI A, LEVI T, SHREM A AND KARPLUS I (1997b). Ration and spatial distribution of feed affect survival, growth, and competition in juvenile red-claw crayfish, *Cherax quadricarinatus*, reared in the laboratory. *Aquaculture* 148(2):169-177. doi: 10.1016/S0044-8486(96)01418-4

BOWSER PR AND ROSEMARK R (1981). Mortalities of cultured lobsters, *Homarus*, associated with a molt death syndrome. *Aquaculture* 23(1-4):11-18. doi: 10.1016/0044-8486(81)90003-X

BUGNOT AB AND LOPEZ GRECO LS (2009). Structural alterations in the male reproductive system of the freshwater crayfish, *Cherax quadricarinatus* (Decapoda, Parastacidae). *J Invertebr Pathol* 102(2):160-166. doi: 10.1016/j.jip.2009.08.004.

CALVO NS, STUMPF L, PIETROKOVSKY S AND LÓPEZ GRECO LS (2011). Early and late effects of feed restriction on survival, growth and hepatopancreas structure in juveniles of the red claw crayfish *Cherax quadricarinatus*. *Aquaculture* 319(3-4):355-362. doi: 10.1016/j.aquaculture.2011.06.033.

CALVO NS, STUMPF L, SACRISTÁN HJ AND LÓPEZ GRECO LS (2013). Energetic reserves and digestive enzyme activities in juveniles of the red claw crayfish *Cherax quadricarinatus* nearby the point-of-no-return. *Aquaculture* 416-417:85-91. doi: 10.1016/j.aquaculture.2013.08.017.

CAMPAÑA-TORRES A, MARTINEZ-CORDOVA LR, VILLARREAL-COLMENARES H AND CIVERA-CERECEDO R (2005). In vivo dry matter and protein digestibility of three plant-derived and four animal-derived feedstuffs and diets for juvenile Australian redclaw, *Cherax quadricarinatus*. *Aquaculture* 250(3-4):748-754. doi: 10.1016/j.aquaculture.2005.02.058.

CAMPAÑA-TORRES A, MARTINEZ-CORDOVA LR, VILLARREAL-COLMENARES H AND CIVERA-CERECEDO R (2008). Carbohydrate and lipid digestibility of animal and vegetal ingredients and diets for the pre-adult redclaw crayfish, *Cherax quadricarinatus* (von Martens). *Aquaculture Research* 39(10):1115-1121. doi: 10.1111/j.1365-2109.2008.01980.x.

CORTÉS-JACINTO E, VILLARREAL-COLMENARES H, CIVERA-CERECEDO R AND MARTÍNEZ-CÓRDOVA R (2003). Effect of dietary protein level on growth and

survival of juvenile freshwater crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *Aquaculture Nutrition* 9(4):207-213. doi: 10.1046/j.1365-2095.2003.00241.x.

DE BOCK MS AND LÓPEZ GRECO LS (2009). Sex reversal and growth performance in juvenile females of the freshwater crayfish *Cherax quadricarinatus* (Parastacidae): effect of increasing temperature and androgenic gland extract in the diet. *Aquaculture International* 18(2):231-243. doi: 10.1007/s10499-008-9239-x.

FAO (2017). *Cherax quadricarinatus* (von Martens, 1868). Cultured species information program. *In:* [online] FAO Fisheries and Aquaculture Department [online].

GARCIA-GUERRERO M, HENDRICKX ME AND VILLARREAL H (2003). Description of the embryonic development of *Cherax quadricarinatus* (Von Martens, 1868) (Decapoda, Parastacidae), based on the staging method. *Crustaceana* 76(3):269-280. doi: 10.1163/156854003765911676.

GARCÍA-GUERRERO M, HERNÁNDEZ-SANDOVAL P, ORDUÑA-ROJAS J AND CORTÉS-JACINTO E (2013). Effect of temperature on weight increase, survival, and thermal preference of juvenile redclaw crayfish *Cherax quadricarinatus*. *Hidrobiológica* 23(1):73-81. doi: not available.

GHANAWI J AND SAOUD IP (2012). Molting, reproductive biology, and hatchery management of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868). *Aquaculture* 358-359:183-195. doi: 10.1016/j.aquaculture.2012.06.019.

JONES C (1990a). The biology and aquaculture potential of the tropical freshwater Crayfish *Cherax quadricarinatus*. *Queensland Department of Primary Industries Information Series* (qi-90028): I-XV, 1-109. doi: not available

JONES C (1995a). Effect of temperature on growth and survival of the tropical freshwater crayfish *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae). *Freshwater Crayfish* 8:391-398. doi: not available

JONES CM (1990b). Biology and aquaculture potential of the tropical Australian freshwater crayfish (*Cherax quadricarinatus*). Pp. 19. Abstracts, Eighth International Symposium of the International Association of Astacology, Baton Rouge, USA, April 22-26, 1990. International Association of Astacology, Baton Rouge.

JONES CM (1990c). The Biology and Aquaculture Potential of the Tropical Freshwater Crayfish, *Cherax quadricarinatus.* QI90028. Department of Primary Industries, Queensland, Brisbane, Australia.

JONES CM (1995b). Effect of temperature on growth and survival of the tropical freshwater crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae). *Freshwater Crayfish* 8:391-398. doi:

JONES CM (1995c). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) I. Development of hatchery and nursery procedures. *Aquaculture* 138(1995):221-238. doi: 10.1016/0044-8486(95)00068-2

JONES CM (1995d). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) II. Juvenile nutrition and habitat. *Aquaculture* 138(1995):239-245. doi: 10.1016/0044-8486(95)00069-0.

JONES CM AND RUSCOE IM (2002). Biological and aquaculture characteristics of five stocks of redclaw, *Cherax quadricarinatus* (von Martens) (Decapoda: Parastacidae) from northern Queensland, Australia Freshwater Crayfish. Pp. 115-135. Proceedings of the Thirteenth Symposium of the International Association of Astacology. doi: not available

JONES CM AND VALVERDE C (2020). Development of Mass Production Hatchery Technology for the Redclaw Crayfish, *Cherax quadricarinatus Freshwater Crayfish* 25(1):1-6. doi: 10.5869/fc.2020.v25-1.001.

KARPLUS I, GIDEON H AND BARKI A (2003). Shifting the natural spring–summer breeding season of the Australian freshwater crayfish *Cherax quadricarinatus* into the winter by environmental manipulations. *Aquaculture* 220(1-4):277-286. doi: 10.1016/s0044-8486(02)00225-9.

KARPLUS I, ZORAN M, MILSTEIN A, HARPAZ S, ERAN Y, JOSEPH D AND SAGI A (1998). Culture of the Australian red-claw crayfish (*Cherax quadricarinatus*) in Israel: III. Survival in earthen ponds under ambient winter temperatures. *Aquaculture* 166(3-4):259-267. doi: 10.1016/S0044-8486(98)00290-7

KING CR (1994). Growth and survival of red claw crayfish hatchlings (*Cherax quadricarinatus* von Martens) in relation to temperature, with comments on the relative suitability of *Cherax quadricarinatus* and *Cherax destructor* for culture in Queensland. *Aquaculture* 122:75-80. doi: 10.1016/0044-8486(94)90335-2

PANIAGUA-CHAVEZ C AND TIERSCH T (2001). Laboratory studies of cryopreservation of sperm and trochophore larvae of the eastern oyster. *Cryobiology* 43(3):211-223. doi: 10.1006/cryo.2001.2346

PRYMACZOK NC, CHAULET A, MEDESANI DA AND RODRÍGUEZ EM (2012). Survival, growth, and physiological responses of advanced juvenile freshwater crayfish (*Cherax quadricarinatus*), reared at low temperature and high salinities. *Aquaculture* 334-337:176-181. doi: 10.1016/j.aquaculture.2011.12.032.

SAMMY N (1988). Breeding biology of *Cherax quadricarinatus* in the Northern *Territory*. Pp. 79-88 *In:* Evans LH and O'Sullivan D, Eds.). Proceedings of the First Australian Aquaculture Conference, Perth, 23-27 October, 1988. Curtin University of Technology, Western Australia. Doi: not available

SAOUD IP, GHANAWI J, THOMPSON KR AND WEBSTER CD (2013). A review of the culture and diseases of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868*). Journal of the World Aquaculture Society* 44(1):1-29. doi: 10.1111/jwas.12011

SAURABH S AND SAHOO P (2008). Major diseases and the defence mechanism in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). *Proceedings of the National Academy of Sciences, India, Section B* 78:103-121. doi: not available

SONG Y, VILLENEUVE DL, TOYOTA K, IGUCHI T AND TOLLEFSEN KE (2017). Ecdysone Receptor Agonism Leading to Lethal Molting Disruption in Arthropods: Review and Adverse Outcome Pathway Development. *Environmental science & technology* 51(8):4142-4157. doi: 10.1021/acs.est.7b00480.

STUMPF L, TROPEA C AND LÓPEZ GRECO LS (2014). Recovery growth of *Cherax quadricarinatus* juveniles fed on two high-protein diets: Effect of daily feeding following a cyclic feeding period on growth, biochemical composition and activity of digestive enzymes. *Aquaculture* 433:404-410. doi: 10.1016/j.aquaculture.2014.06.037.

VILLARREAL H (1991). A partial energy budget for the Australian crayfish *Cherax tenuimanus*. *Journal of the World Aquaculture Society* 22(4):252-259. doi: 10.1111/j.1749-7345.1991.tb00742.x

WEBSTER CD, THOMPSON KR, MUZINIC LA, YANCEY DH, DASGUPTA S, XIONG YL, ROUSE DB AND MANOMAITIS L (2004). A preliminary assessment of growth, survival, yield, and economic return of Australian red claw crayfish, *Cherax quadricarinatus*, stocked at three densities in earthen ponds in a cool, temperate climate. *Journal of Applied Aquaculture* 15(3-4):37-50. doi: 10.1300/J028v15n03_03.

YEH HS AND ROUSE DB (1995). Effects of water temperature, density, and sex ratio on the spawning rate of red claw crayfish *Cherax quadricarinatus* (von Martens). *Journal of the World Aquaculture Society* 26(2):160-164. doi: 10.1111/j.1749-7345.1995.tb00239.x

ZHAO Y, MENG F, CHEN L, GU Z, XU G AND LIU Q (2000). Effects of Different Gradient Temperatures on Embryonic Development of the *Cherax quadricarinatus* (Crustacea, Decapoda). *Journal of Lake Sciences* 12(1):59-62. doi: not available.

ZHU H, JIANG Q, WANG Q, YANG J, DONG S AND YANG J (2013). Effect of dietary carbohydrate-to-lipid ratios on growth performance, body composition, hepatic enzyme activities, and digestive enzyme activities of juvenile Australian Redclaw Crayfish, *Cherax quadricarinatus* (von Martens). *Journal of the World Aquaculture Society* 44(2):173-186. doi: 10.1111/jwas.12024.

Chapter 4: Morphology and Weight-length Relationships for the First Six Instars of *Cherax quadricarinatus* (von Martens, 1868)

A Version of this Chapter has been published as:

Rigg DP, Courtney RL, Jones CM and Seymour JE (2021). Morphology and Weight-length Relationships for the First Six Instars of *Cherax quadricarinatus* (von Martens, 1868). Freshwater Crayfish 26(1): 1-8. **doi:** Not Yet Available.

Statement of the Contribution of Others

Damian Rigg is the primary author of this chapter and was extensively involved in all aspects of this work under the supervision of: Associate Professor Clive Jones, Professor Jamie Seymour and Dr. Robert Courtney. Jessica Sleeman and Sally Browning assisted with animal husbandry; numerous aquarium volunteers also assisted with animal husbandry. Financial support was supplied by AgriFutures Australia (formerly RIRDC) which provided all necessary equipment and funding for travel and conference attendance. The research was supported by the North Queensland Crayfish Farmer's Association and AquaVerde Redclaw Crayfish Farm and Hatchery which supplied experimental animals and broodstock (www.AquaVerde.com.au), grateful thanks to Colin and Ursula Valverde. The Australian Postgraduate Award (APA) scholarship provided financial support for Damian Rigg during this candidature. We thank the Freshwater Crayfish editor and the two anonymous reviewers for their useful comments and suggestions.

Chapter 4: Morphology and Weight-length Relationships for the First Six Instars of *Cherax quadricarinatus* (von Martens, 1868)

4.1 Abstract

Cherax quadricarinatus (redclaw) aquaculture has not achieved the industry growth that had been predicted in the early days of development. Provision of quality juvenile crayfish seed stock has been identified as a critical factor in overcoming industry development inertia. Hatchery technology has been developed to produce independent craylings and a nursery phase is now being developed to nurture the delicate craylings to a more advanced and robust juvenile stage, suitable for pond stocking. As part of the nursery development, clear definitions of the successive stages from egg through the first several instars, are required. Although some morphological descriptions of the early stages of redclaw have been made, the characteristics and nomenclature for stages from egg through to an advanced juvenile need clarification. A naming system is proposed (Egg, L1, L2, J1, J2 and J3) for the first six instars from egg, based on gross morphology and allometric relationships. Egg volume, wet weight and ocular carapace length (OCL) were analysed through linear regression. Based on these variables, the size of each instar is defined. Descriptions and photographs of the six instars provide a visual reference for identification. Five of the six instars had a significant relationship between wet weight and OCL. Both significantly increased for each successive instar. Lyophilized (dry) weight was not significantly different between stages until after instar J1 where endogenous feeding begins. The growth of the first four instars in terms of wet weight and OCL, but not dry weight, suggests an extra endogenous source of nutrition in addition to the yolk supply. Branchial water uptake during ecdysis has been proposed as a route for dissolved organic matter or small

particulate matter to be acquired by the crayfish during the lecithotrophic, non-exogenous feeding stages as an additional food source that might further enhance growth and survival.

4.2 Introduction

The redclaw crayfish *Cherax quadricarinatus* is a native species originating from rivers and creeks which drain into the Gulf of Carpentaria, bordering the Australian states of Queensland and the Northern Territory (Jones 1990). In the 1980's redclaw was introduced into aquaculture but since then has failed to develop into the thriving industry which was expected. One of the areas identified that might support industry growth is the development of hatchery and nursery phases for production of seed stock. In order to develop standard operating procedures for these phases, more information is required on the early instars, for which knowledge is currently incomplete.

The embryonic development of redclaw within the egg through to hatching is well documented (King 1993; Yeh and Rouse 1994; Parnes and Sagi 2002; Garcia-Guerrero *et al*. 2003), however the post-embryonic instars from the first hatched instar through to the free-living instars are less well described. In particular the instars which would be involved in a nursery phase need to be fully described in terms of morphology, weight and size. Although the first two post-hatch instars have been described (Loya-Javellana *et al*. 1993; Jones 1995; Levi *et al*. 1999; Garcia-Guerrero *et al*. 2003) the subsequent stages from post-hatch instar 3 have not been described in the scientific literature. Furthermore, there is some confusion in the literature about the naming convention for instars from egg through hatching and the first four post-hatch moults, with authors using variable nomenclature for these instars. Loya-Javellana (1993)

referred to the first three hatched instars as Independent Stage I with yolk present, Independent Stage I without obvious yolk, and Independent Stage II, whereas Levi *et al*. (1999) named them as young of Stage 1, 2 or 3. The descriptions of the first three post-hatch instars by Levi *et al*. (1999) however provide good reference in terms of identification. In addition to morphological descriptions, there is a gap in the literature concerning the bivariate relationships of length and weight for all the early instars. For a complete developmental description, it is necessary to quantify these relationships from egg through the next five instars to include those stages likely to be involved in a nursery phase. In this paper we present an alternate nomenclature for the first six instars in redclaw ontogeny, to add clarification and continuity to the naming system and quantitative descriptions of each.

The aims of this study were firstly to develop a logical sequential nomenclature with morphological descriptions of the first six instars of *C. quadricarinatus* to assist in identification of stages for a nursery phase. Secondly, we examined the bivariate relationships between ocular carapace length (OCL) and weight (wet weight and lyophilized dry weight) for the first six instars and determined the mean lengths and weights for each. These data were then used to consider changes in morphology in relation to habitat and ecology. Finally, we examined the data to discover whether they contain information which may assist in enhancing aquaculture of the early life stages.

4.3 Materials and Methods

4.3.1 Source of Crayfish

Redclaw eggs sourced from Aquaverde Redclaw Farm and hatchery (17.3090°S, 145.4593°E, aquaverde.com.au) were stripped from broodstock females (held at 27°C - 28°C) at an advanced stage when eyes were visible in the eggs and either

shipped to James Cook University (Cairns Campus) (JCU) for experiments or hatched in the Aquaverde incubator at a temperature of 26°C +/- 1°C (Jones and Valverde 2020). Eggs which were sourced from the broodstock facility held at JCU Aquarium were stripped from broodstock females (held at 27°C - 28°C) at the same advanced stage with eyes visible. Crayfish on-grown to successive instars in the laboratory at JCU were all held at 24°C +/- 1°C in discrete aerated recirculating systems consisting of two tanks of 8 L each and a 10 L sump, total system volume of 26 L, and a flow rate of 60 L H^{-1} per tank. A 20 L water exchange was performed each day using aged aerated tap water. All instars were held *en masse* in the tanks, habitat was provided in the form of a bundle of dressmaker's tulle. Water quality parameters were maintained at: pH between 6.6 and 7.6, ammonia between 0 and 0.5 ppm, nitrite at 0 ppm, nitrate between 0 and 10 ppm. All redclaw in the endogenous feeding instars were fed defrosted frozen enriched *Artemia* sp. to satiation.

4.3.2 Naming of the Instars

The first six instars of redclaw *C. quadricarinatus* were designated in chronological order, with descriptions from the literature for instars 1 – 4 (Table 4). Instars 5 and 6 have not previously been described and instars from J1 (instar 4) generally are referred to as juvenile in the literature. The stages noted here as J1, J2 and J3 are nominal and used simply to define successive stages between moults from J1 stage where the crayfish assume their adult morphology. In chronological order the instars are; Egg, L1, L2, J1, J2 and J3.

Table 4. Naming of first 6 instars of *Cherax quadricarinatus*.

Instar	Name	Other names from the literature
1	Egg prior to hatching	• Stage 5 – 6 (Jones 1995) • Stage 7 (Yeh and Rouse 1994) • Stage 8, 80% development (Garcia-Guerrero *et al*. 2003)
2	L1 (larval stage 1)	• Stage 7 hatched and attached (Jones 1995) • Hatchling (King 1993) • Stage 8, 1 of 3 larval stages (Yeh and Rouse 1994) • Stage 2 crayling (Parnes and Sagi 2002) • Stage 11, post-embryo I (Garcia-Guerrero *et al*. 2003)
3	L2 (larval stage 2)	• Stage 7 hatched and attached (Jones 1995) • Hatchling (King 1993) • Stage 8, 1 of 3 larval stages (Yeh and Rouse 1994) • Stage 12, post-embryo II (Garcia-Guerrero *et al*. 2003)
4	J1 (juvenile stage 1)	• Hatchling (King 1993) • Stage 9, free-swimming juvenile (Yeh and Rouse 1994) • Juvenile (Parnes and Sagi 2002; Garcia-Guerrero *et al*. 2003) • Stage 3 juvenile S3J (Stevenson *et al*. 2013).
5	J2 (juvenile stage 2)	• Not previously described
6	J3 (juvenile stage 3)	• Not previously described

4.3.3 Photography

A sample of each instar was photographed using a Nikon digital SLR D810 camera coupled to a dissecting microscope (Olympus SZ40). Crayfish were individually placed on a 1mm x 1mm graticule to illustrate comparative size between instars, and the features pertaining to each instar such as differences in body morphology were also described.

4.3.4 Statistical Analyses

A linear regression analysis was performed to examine the bivariate relationship between ocular carapace length (OCL) or volume for eggs and wet weight for each instar. A one-way ANOVA with *post hoc* LSD was performed on OCL for instars L1 to J3 (eggs were excluded on the basis that they had a volume measurement instead of OCL), a one-way ANOVA with *post hoc* LSD was performed on wet weight for instars egg to J3, and a one-way ANOVA with *post hoc* LSD was performed on lyophilized dry weight for instars egg to J3. Wet weight, OCL and lyophilized dry weight means were calculated for each instar. All statistical analyses were performed using IBM SPSS Statistics version 25.

4.3.5 Weight and Volume

Redclaw eggs and all of the next five instars, inclusive of L1 to J3, were placed on absorbent paper for 3 seconds to remove exterior water and weighed on a four-point balance. Eggs were measured across the long and short axes and the egg volume calculated from these data using the ellipsoid volumetric formula as per Koc (2007). The subsequent instars were measured for OCL on a 1 mm graticule, measured from behind the ocular cavity to the posterior margin of the cephalothorax (Plate 1). All instars were placed in -80°C freezer for storage, followed by lyophilization for a minimum of 3 days, then weighed to determine lyophilized, dry weight.

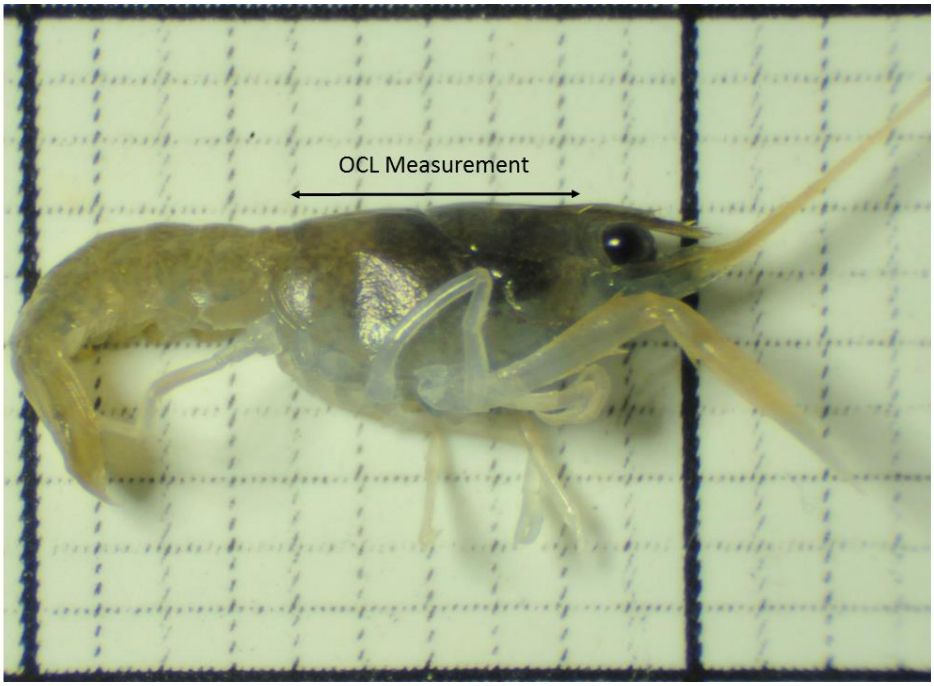

Plate 1. Ocular carapace length measurement for *Cherax quadricarinatus.*

4.4 Results

4.4.1 Morphology of Early Life Instars of *C. quadricarinatus*

Late stage eggs show the eyes clearly visible and differentiation and structure of the embryo within the egg (Plate 2.A). L1 instar shows an early body form, largely undifferentiated between the cephalothorax and almost transparent abdomen, with an integral yolk sac (Plate 2.B). After the first ecdysis, instar L2 exhibits a more differentiated body form with reduction of the yolk sac, and eye stalks and a rostrum developing (Plate 2.C). Following the second ecdysis the J1 has a depleted yolk sac which allows for the cephalothorax and abdomen to achieve the shape and proportions of the adult crayfish, and pigmentation has begun (Plate 2.D). Post third ecdysis, instar J2 (Plate 2.E) and post fourth ecdysis instar J3 (Plate 2.F) are essentially indistinguishable from instar J1, apart from an increase in size and mass.

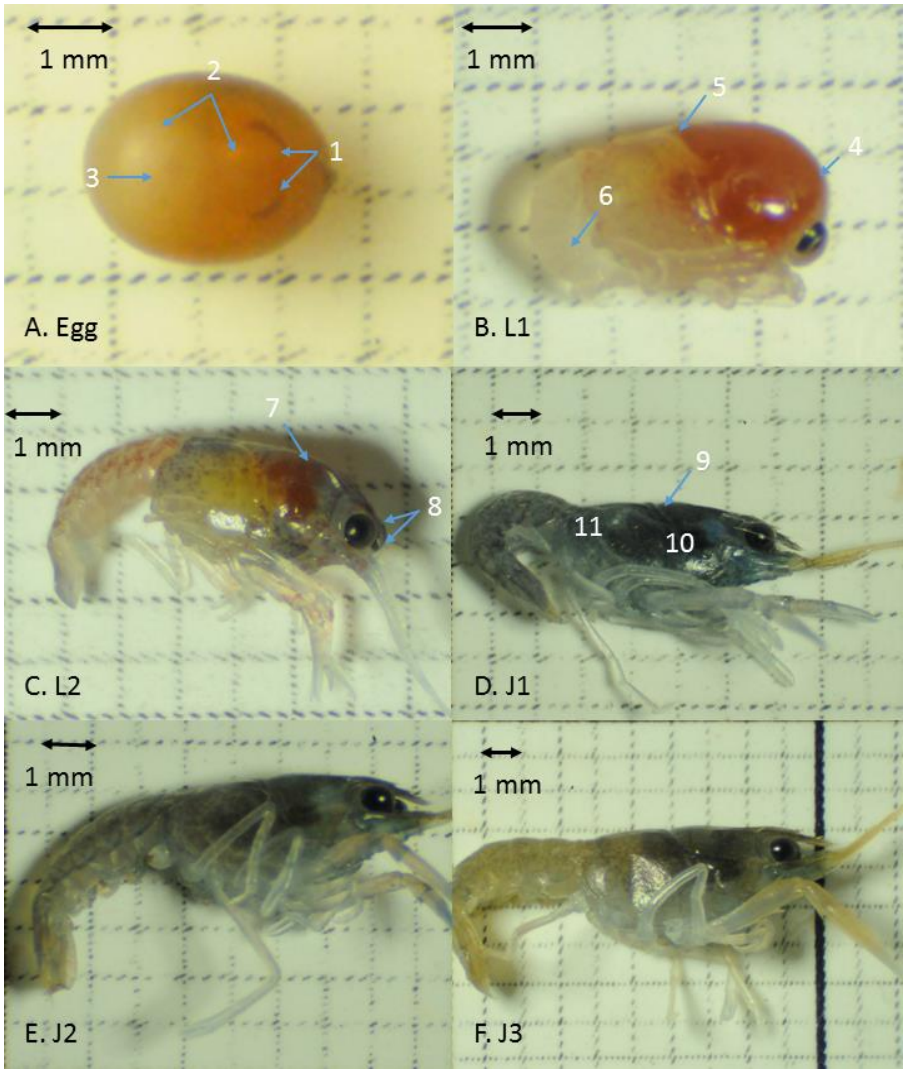

Plate 2. Photographs of the first six instars of *Cherax quadricarinatus*.
(A) *C. quadricarinatus* late stage egg showing crescent-shaped eyes (1), differentiation within the egg (2) a translucent area posterior to the eyes (3) and a general granular appearance of egg contents. (B) *C. quadricarinatus* L1 instar, a non-mobile lecithotrophic stage, showing the large, rounded cephalothorax (4), largely undifferentiated abdomen to carapace region with a hunchback containing yolk (5), and an almost transparent abdomen (6). (C) *C. quadricarinatus* L2 instar, the final lecithotrophic stage, showing differentiation from the L1 stage via reduction in the shape and size of the hunchback due to partial depletion of the yolk sac (7), and commencement of the development of the eye-stalks and rostrum (8). (D) *C. quadricarinatus* J1 instar is the first instar with independent locomotion and exogenous feeding, has a depletion of the yolk sac resulting in the disappearance of the hunchback form (9), and the appearance of the cephalothorax in the proportions and shape which will continue throughout the life of the crayfish (10). The J1 instar also has the beginnings of darker pigmentation (11). (E) *C. quadricarinatus* J2 instar, indiscernible from J1 instar except for increase in size and mass. (F) *C. quadricarinatus* J3 instar, also indiscernible from J1 or J2 instar except for an increase in size and mass.

4.4.2 Bivariate Relationship between OCL and Wet Weight for Early *C. quadricarinatus* instars

There was a significant positive relationship between wet weight and volume for *C. quadricarinatus* eggs, [Wet Weight (g) = 1.01E-3 + 1.22E-4 x Volume (mm^3)], (R^2 = .611, $F_{1, 189}$ = 296.614, p < 0.001), (Figure 10).

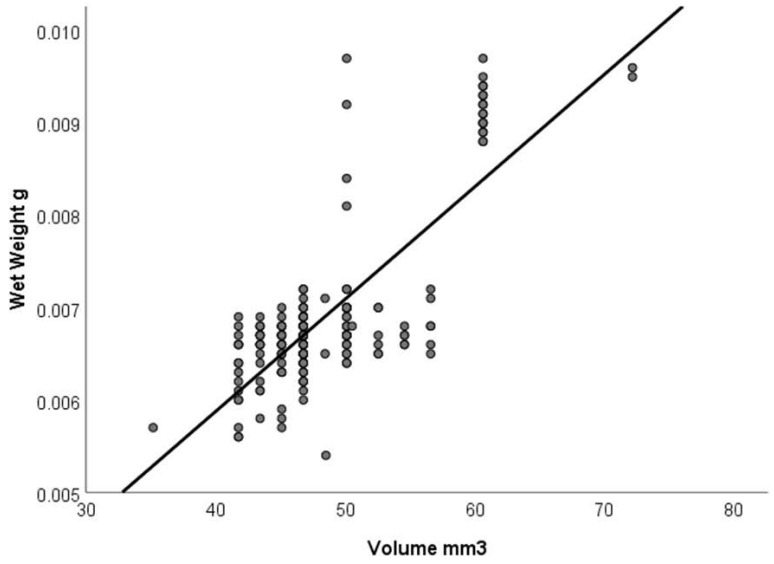

Figure 10. Bivariate relationship for *Cherax quadricarinatus* eggs, the predictor is individual egg volume, the response variable is individual wet weight.

There was no significant relationship between wet weight and OCL for instar L1 [Wet Weight (g) = 1.71E-4 x OCL (mm) + 1.18E-2], (R^2 = .001, $F_{1, 167}$ = 0.191, p = 0.663). There was a significant positive relationship between wet weight and OCL for instar L2, however only 9% of the variation was explained, [Wet Weight (g) = 1.66E-3 x OCL (mm) + 8.88E-3], (R^2 = .086 $F_{1, 176}$ = 13.363, p < 0.001), (Figure 11).

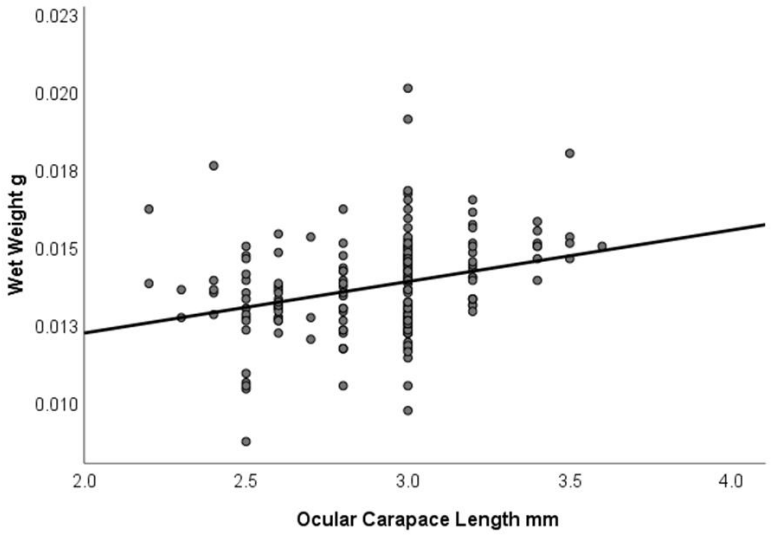

Figure 11. Bivariate relationship for *Cherax quadricarinatus* L2 instar, the predictor is individual ocular carapace length, the response variable is individual wet weight.

There was a significant positive relationship between wet weight and OCL for instar J1 despite only 4% of the variation being explained [Wet Weight (g) = 1.86E-3 x OCL (mm) + 1.25E-2], (R^2 = .040, $F_{1, 194}$ = 7.948, p = 0.005), (Figure 12).

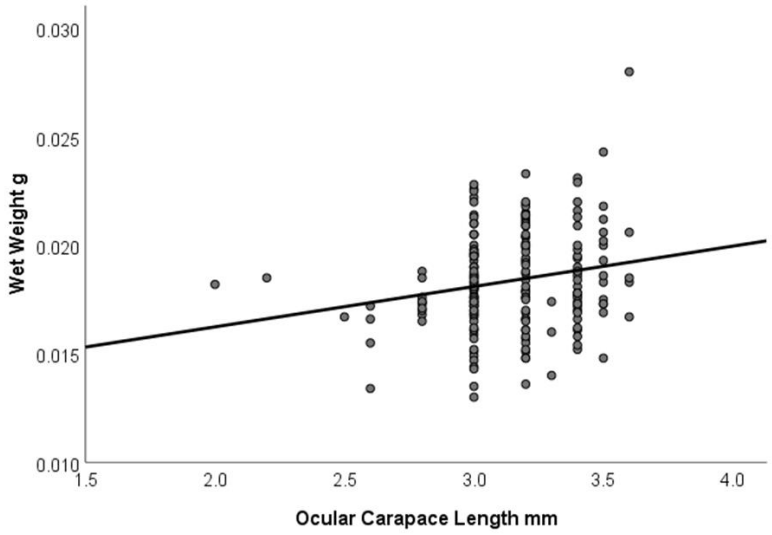

Figure 12. Bivariate relationship for *Cherax quadricarinatus* J1 instar, the predictor is ocular carapace length, the response variable is individual wet weight.

There was a significant positive relationship for *C. quadricarinatus* J2 instar between wet weight and OCL [Individual Wet Weight (g) = 1.79E-2 x OCL (mm) +- 3.86E-2], (R^2 = .711, $F_{1, 190}$ = 467.944, p < 0.001), (Figure 13).

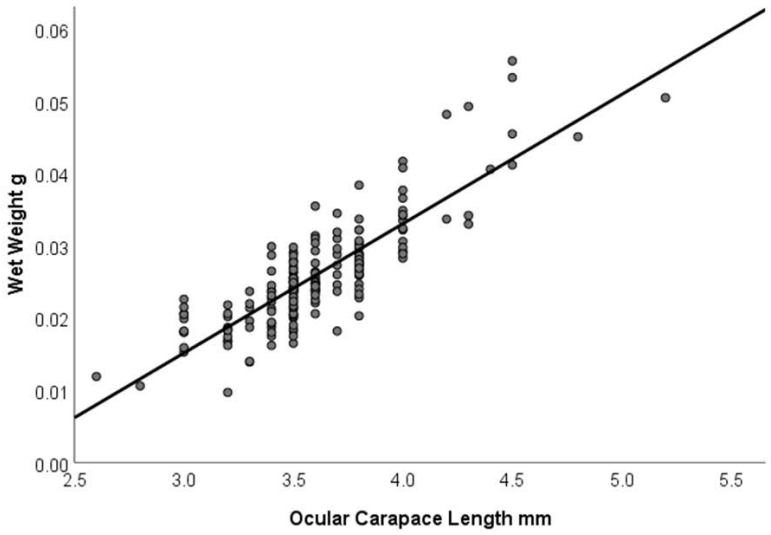

Figure 13. Bivariate relationship for *Cherax quadricarinatus* J2 instar, the predictor is individual ocular carapace length, the response variable is individual wet weight.

There was a significant positive relationship for *C. quadricarinatus* J3 instar between wet weight and OCL [Individual Wet Weight (g) = 2.36E-2 x OCL (mm) + -5.48E-2], (R^2 = .681, $F_{1, 208}$ = 441.690, p < 0.001), (Figure 14).

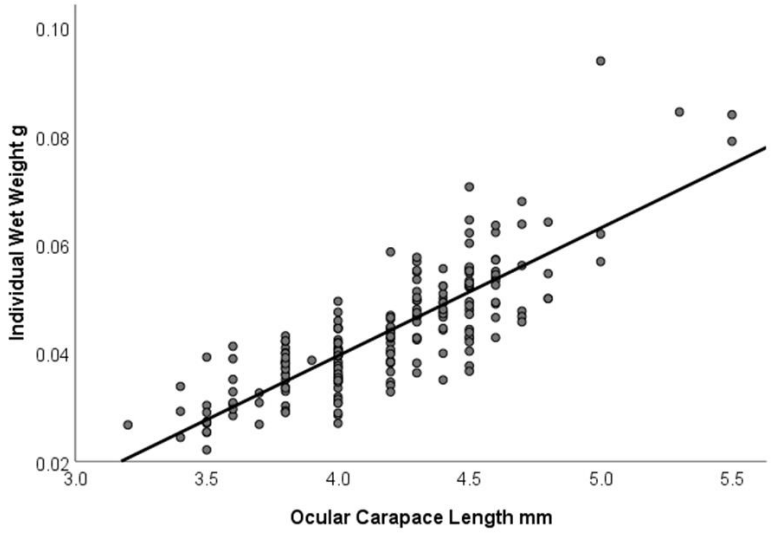

Figure 14. Bivariate relationship for *Cherax quadricarinatus* J3 instar, the predictor is individual ocular carapace length, the response variable is individual wet weight.

4.4.3 Comparative Relationships for Early Instars of *C. quadricarinatus*

There was a statistically significant difference in the OCL between the life stages with an increase in OCL over consecutive instars, $F_{4, 937} = 677.256$, $p < 0.001$ (Figure 15).

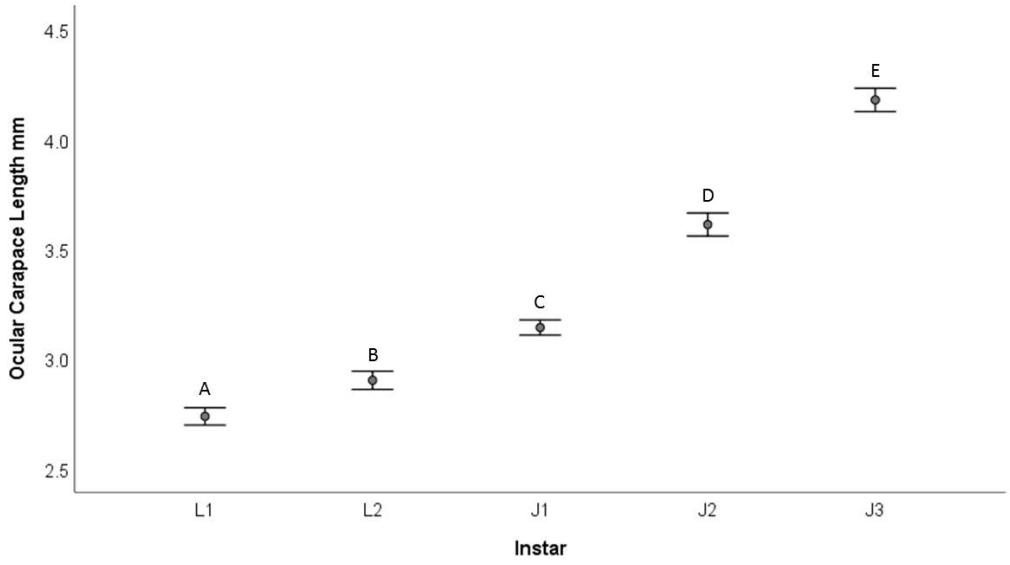

Figure 15. OCL means for the first five hatched instars of redclaw *Cherax quadricarinatus.*
Error bars represent 95% CI, bars with the same letter are not significantly different.

Mean wet weight for each instar is significantly different and increases with increasing age $F_{5, 1640} = 1436.238$, $p < 0.001$ (Figure 16).

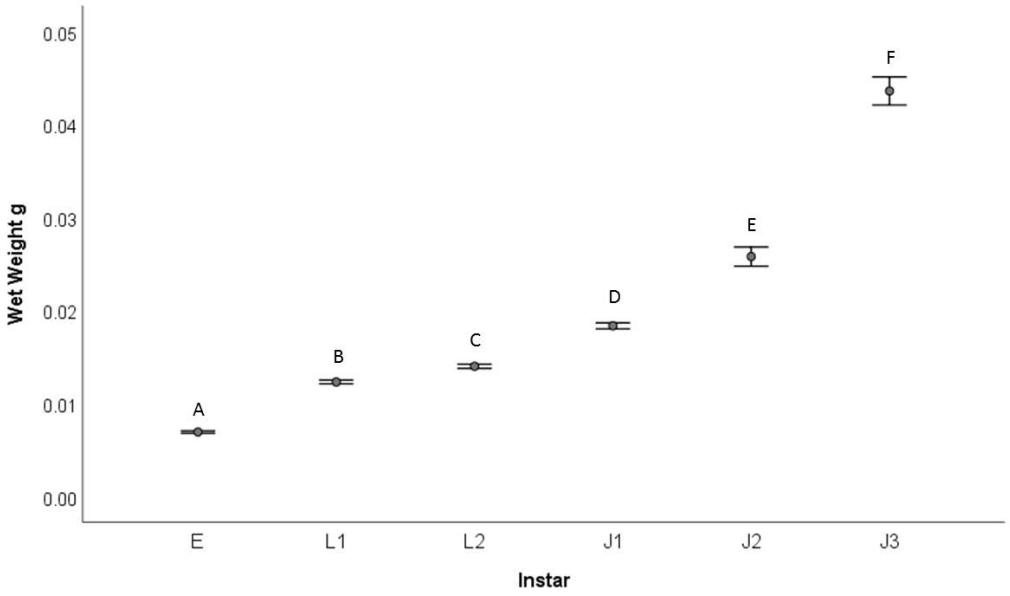

Figure 16. Wet weight means for the first six instars of redclaw *Cherax quadricarinatus*.
Error bars represent 95% CI, bars with the same letter are not significantly different.

The lyophilized dry weight of *C. quadricarinatus* did not change significantly between instars from egg to J1, however there is an increase in lyophilized dry weight at the J2 size which is significantly larger than egg, L1, L2 and J1, $F_{5, 1127} = 431.268$, $p < 0.001$ (Figure 17). Similarly, J3 crayfish are significantly larger in terms of lyophilized dry weight than all the previous instars (Figure 17).

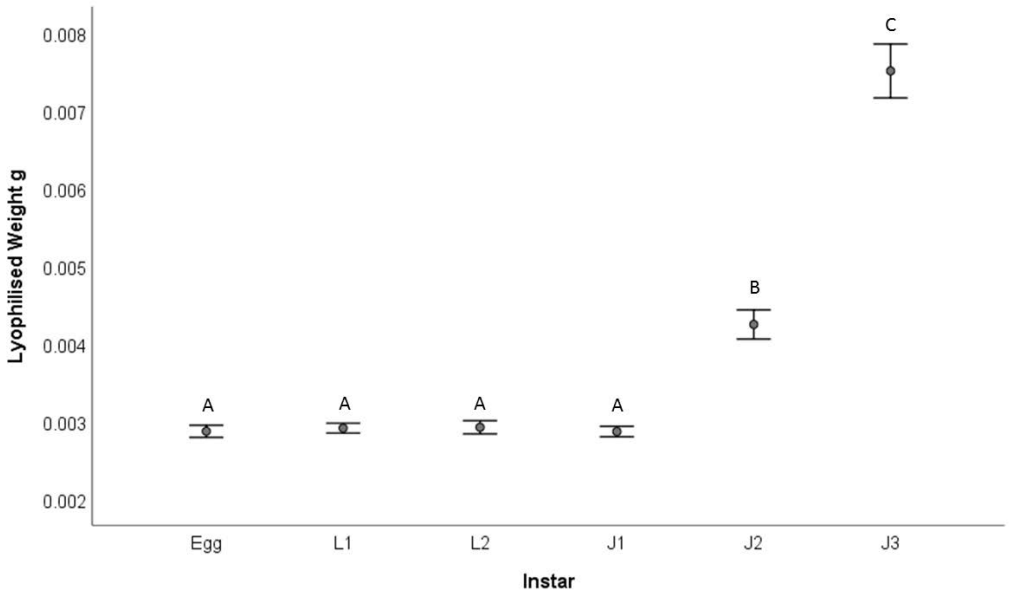

Figure 17. Lyophilized dry weight means for the first six instars of redclaw *Cherax quadricarinatus.*
Error bars represent 95% CI. Means with the same letter are not significantly different.

Wet weight, OCL and lyophilized dry weight means were calculated for each of the first six instars for *C. quadricarinatus* (Table 5). Wet weight increased from egg to L1, and progressively through the instars to J3 (Figure 16, Table 5). OCL also showed a progressive increase from L1 to J3, whereas lyophilized dry weight did not change until J2 instar (Figure 15, Figure 17, and Table 5).

Table 5. Mean weight and length data for the first six instars of *Cherax quadricarinatus.*

Instar		Wet Weight (g)	OCL (mm) Or Egg Volume (mm^3)	Lyophilized Dry Weight (g)
Egg	*n*	192	191	163
	Mean	0.006920	48.4985	0.002874
L1	*n*	174	169	163
	Mean	0.012303	2.7349	0.002915
L2	*n*	253	177	230
	Mean	0.013989	2.8994	0.002926
J1	*n*	197	195	185
	Mean	0.018339	3.1405	0.002871
J2	*n*	197	192	194
	Mean	0.025786	3.6005	0.004248
J3	*n*	215	209	198
	Mean	0.043601	4.1789	0.007507

Although instars L2, J1, J2 and J3 have significant wet weight / OCL relationships that enable wet weight to be accurately estimated from an OCL measurement, neither OCL nor wet weight measurements are sufficient to unambiguously identify a particular stage. Given the variability in wet weight due to physiological and nutritional factors, OCL is considered the most reliable predictor of the stage as it is less variable than wet weight.

4.5 Discussion

Although the first four instars of *C. quadricarinatus* have been photographed and described before (King 1993; Yeh and Rouse 1994; Jones 1995; Parnes and Sagi 2002; Garcia-Guerrero *et al*. 2003; Stevenson 2013), the descriptions of the subsequent instars that would likely be included in a nursery phase, i.e. J2 and J3, provide important data for aquaculture purposes (Plate 2). The morphological description of instars J2 and J3, also highlights the difficulty of accurate identification of these instars. As the morphology of J2 and J3 is equivalent to J1, only weight and OCL data can be used to

distinguish between stages. Even then, unambiguous identification of stage by wet weight or OCL is not possible with certainty due to the overlapping variance of each mean. Further still, the means for wet weight and OCL for any given stage will vary according to genetic stock and environmental and nutritional factors.

For five of the six instars examined, there was a significant relationship between wet weight and OCL which allows for computation of the wet weight by using the equation for the regression function if OCL is known. Combining wet weight and OCL data with morphology will enable accurate identification of the first three instars for *C. quadricarinatus*. However, for the subsequent three stages weight and / or OCL provide only a guide to the stage.

If a nursery phase is to be applied in the aquaculture of redclaw, accurate identification of the developmental stage will be important. According to industry experience (Jones and Valverde 2020) the initial moults from L1 to L2 and L2 to J1 are quite synchronous. However, they subsequently become less synchronised, so that a cohort becomes a mix of different instars. Using morphological and size data to accurately identify the developmental stage of individual crayfish could assist nursery farmers to identify the more advanced individuals, best suited for pond stocking. The information here provides a valuable aid in identifying and potentially discarding inferior crayfish that are developing more slowly, to enable selection of the fittest and fastest growing animals for grow-out pond stocking. Although differentiating between J1, J2 and J3 is difficult, a single cohort of redclaw grown under the same conditions will show some obvious differences in rate of development, and as such the smallest animals can be separated and removed regardless of the instar. Such interventions early on may have a positive effect on subsequent survival and growth to harvest.

Although further allometric examination of the early instars might identify other dimensions where disproportionate changes between instars would enable discrete identification, such as leg or antennae length, relative to body size, these would be of limited practical value to commercial nursery farmers. At this time, it seems body size as measured by wet weight or OCL is the most practical metric that enables reasonably accurate identification of instar. Practical application would be best applied on a collective cohort basis, whereby the mean size (wet weight or OCL) of a substantial sample of the cohort is determined and this datum used to determine the likely instar, based on the data presented in Table 5.

The early lecithotrophic instars of *C. quadricarinatus* provide some interesting data on the mechanisms of growth and the apparent use of a food source exogenous to the growing crayfish. Although the instars from egg to J1 are considered lecithotrophic and essentially sealed from the outside environment (Garcia-Guerrero *et al*. 2003), OCL increases significantly with each successive instar from egg (Figure 15), wet weight increases significantly with each successive instar from egg (Figure 16), and lyophilised dry weight remains unchanged between egg and J1, increasing significantly at J2 and at J3 (Figure 17). With no apparent nutritional input from the external environment, the process of growth in size (OCL) and weight (both wet and lyophilised dry weight) requires energy, sourced from the stored yolk. Use of the finite yolk supply for metabolism and somatic growth without any other energy source should result in an overall loss of mass from stage to stage. Since this is not evident, it suggests an exogenous energy source may be involved.

Water uptake during ecdysis is functionally complicated (Wheatly and Ayers 1995) and involves many chemical and physical processes (Shechter *et al*. 2008), however the potential for nutrient uptake within the water used to swell the animal and

through the rupturing of the thoracoabdominal membranes gives the exogenous water access to exuvial fluids in the apolysis space (Phlippen *et al*. 2000) and a potential pathway for uptake of dissolved or suspended organic matter. Branchial uptake of water from the surrounding environment has been shown as one of the pathways for restoring mineral balance in freshwater crayfish lost through ecdysis, especially in smaller animals (Wheatly and Ayers 1995; Shechter *et al*. 2008). The uptake of dissolved organic matter (DOM) and / or microparticle exogenous food sources by small freshwater lacustrine crustaceans has shown evidence of the contribution to their energy requirements (Salonen and Hammar 1986; Kankaala *et al*. 2010). The water uptake in this instance around the rupturing and hatching of the eggs as well as during ecdysis may be the direct pathway by which DOM / microparticle food sources can be obtained as a supplementary or additional food source during the lecithotrophic instars and therefore may provide a way to enhance the very early instars of redclaw. If the crayfish are receiving nutrition for growth from the external environment, through DOM /microparticle uptake, a possible way to enhance nutrition and perhaps growth and survivorship is to determine how to add the essential nutrients to the water so that they can be assimilated.

4.6 Conclusions and Future Directions

Data gathered and presented here will contribute to the definition of standard operating procedures for a redclaw nursery phase, through identification of the instar through descriptive morphology and size (OCL or wet weight). Having such metrics for the instars allows very early grading into specific instars, allowing for exclusion of slow-growing or small animals, thus ensuring that only the best and fittest animals are stocked after the nursery phase. More data is required on the weights and sizes of the

first six instars for different redclaw genetic stocks and animals reared under different temperature and feeding regimes to compare with data presented here and further assist in distinguishing between J1, J2 and J3. Further allometric examination is also warranted to identify other body dimensions, such as antennae or leg length, which may display differential growth that enables instars to be more precisely differentiated.

When combined with other factors such as diet from J1 onwards, the most suitable temperature for each instar, and the provision of habitat or shelter, a nursery phase designed around these components may assist the redclaw aquaculture industry in overcoming the major obstacle to investment being high variability in harvest. Furthermore, a study to examine the physical processes involved with how DOM or microparticulate uptake is occurring and which nutrients the crayfish are absorbing may provide an opportunity to enhance their size and weight and produce fitter, stronger crayfish better able to survive to harvest in aquaculture.

4.7 Literature Cited

GARCIA-GUERRERO M, HENDRICKX ME AND VILLARREAL H (2003). Description of the embryonic development of *Cherax quadricarinatus* (Von Martens, 1868) (Decapoda, Parastacidae), based on the staging method. *Crustaceana* V76 (3):269-280. doi: 10.1163/156854003765911676.

JONES C (1995). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) .1. Development of hatchery and nursery procedures. *Aquaculture* 138(1-4):221-238. doi: 10.1016/0044-8486(95)00068-2.

JONES CM (1990). Biology and aquaculture potential of the tropical Australian freshwater crayfish (*Cherax quadricarinatus*). Pp. 19. Abstracts, Eighth International Symposium of the International Association of Astacology, Baton Rouge, USA, April 22-26, 1990. International Association of Astacology, Baton Rouge. doi: not available.

JONES CM AND VALVERDE C (2020). Development of Mass Production Hatchery Technology for the Redclaw Crayfish, *Cherax quadricarinatus Freshwater Crayfish* 25(1):1-6. doi: 10.5869/fc.2020.v25-1.001.

KANKAALA P, TAIPALE S, LI L AND JONES RI (2010). Diets of crustacean zooplankton, inferred from stable carbon and nitrogen isotope analyses, in lakes with varying allochthonous dissolved organic carbon content. *Aquatic Ecology* 44(4):781-795. doi: 10.1007/s10452-010-9316-x.

KING CR (1993). Egg development time and storage for redclaw crayfish *Cherax quadricarinatus* von Martens. *Aquaculture* 109:275-280. doi: 10.1016/0044-8486(93)90169-Y.

KOC AB (2007). Determination of watermelon volume using ellipsoid approximation and image processing. . *Postharvest Biology and Technology* 45(3):366-371. doi: 10.1016/j.postharvbio.2007.03.010.

LEVI T, BARKI A, HULATA G AND KARPLUS I (1999). Mother-offspring relationships in the red-claw crayfish *Cherax quadricarinatus*. *Journal of Crustacean Biology* 19(3):477-484. doi: 10.2307/1549256.

LOYA-JAVELLANA GN, FIELDER DR AND THORNE MJ (1993). Food choice by free-living stages of the tropical freshwater crayfish, *Cherax quadricarinatus* (Parastacidae: Decapoda). *Aquaculture* 118(3):299-308. doi: 10.1016/0044-8486(93)90464-A.

PARNES S AND SAGI A (2002). Intensification of redclaw crayfish *Cherax quadricarinatus* culture: I. Hatchery and nursery system. *Aquacultural Engineering* 26(4):251-262. doi: 10.1016/S0144-8609(02)00034-1

PHLIPPEN MK, WEBSTER SG, CHUNG JS AND DIRCKSEN H (2000). Ecdysis of decapod crustaceans is associated with a dramatic release of crustacean cardioactive peptide into the haemolymph. *Journal of Experimental Biology* 203(3):521-536. doi: not available.

SALONEN K AND HAMMAR T (1986). On the importance of dissolved organic matter in the nutrition of zooplankton in some lake waters. *Oecologia* 68(2):246-253. doi: 10.1007/BF00384795

SHECHTER A, BERMAN A, SINGER A, FREIMAN A, GRINSTEIN M, EREZ J, AFLALO ED AND SAGI A (2008). Reciprocal changes in calcification of the gastrolith and cuticle during the molt cycle of the red claw crayfish *Cherax quadricarinatus*. *The Biological Bulletin* 214(2):122-134. doi: not available.

STEVENSON J, JERRY D AND OWENS L (2013). Redclaw Selective Breeding Project. RIRDC Publication No. 13/007 RIRDC Project No. PRJ-000327 Final Report. Rural Industries Research and Development Corporation, Canberra, pp. 47. doi: not available.

WHEATLY MG AND AYERS J (1995). Scaling of calcium, inorganic contents, and organic contents to body mass during the molting cycle of the fresh-water crayfish *Procambarus clarkii* (Girard). *Journal of Crustacean Biology* 15(3):409-417. doi: 10.2307/1548762

YEH H-S AND ROUSE DB (1994). Indoor spawning and egg development of the red claw crayfish *Cherax quadricarinatus*. *Journal of the World Aquaculture Society* 25(2):297-302. doi: 10.1111/j.1749-7345.1994.tb00194.x.

Chapter 5. Identifying Suitable Hatchery and Nursery Phase Thermal Regimes for the Early Life Instars of Redclaw Crayfish *Cherax quadricarinatus* (von Martens, 1868) through Examination of the Effects of Temperature upon Oxygen Consumption.

A Version of this Chapter has been submitted to Freshwater Crayfish Journal as below, and is currently under review:

Rigg DP, Courtney RL, Jones CM and Seymour JE (2021). Identifying Suitable Hatchery and Nursery Phase Thermal Regimes for the Early Life Instars of Redclaw Crayfish *Cherax quadricarinatus* (von Martens, 1868) through Examination of the Effects of Temperature upon Oxygen Consumption.

Statement of the Contribution of Others

Damian Rigg is the primary author of this chapter and was extensively involved in all aspects of this work under the supervision of: Associate Professor Clive Jones, Professor Jamie Seymour and Dr. Robert Courtney. Professor Jamie Seymour and Dr. Robert Courtney supplied and developed use of the respirometry equipment and associated protocols. Jessica Sleeman and Sally Browning assisted with animal husbandry, numerous aquarium volunteers also assisted with animal husbandry. Financial project support was supplied by AgriFutures Australia (formerly RIRDC). The research was supported by the North Queensland Crayfish Farmer's Association and AquaVerde Redclaw Crayfish Farm and Hatchery which supplied experimental animals and broodstock (www.AquaVerde.com.au), grateful thanks to Colin and Ursula Valverde. The Australian Postgraduate Award (APA) scholarship provided financial support for Damian Rigg during this candidature.

5.1 Abstract

The main barrier to the development of a large-scale aquaculture industry for redclaw appears to be the variability in survival at the commercial aquaculture grow-out level which may be caused by survivorship of the early life stages. Culture temperature is of utmost importance and may be one aspect that causes mortality. Previous research suggests younger stages require lower temperature for survival than advanced juveniles and adults. Egg thermal regimes have been suggested as 22°C – 25°C, J1 instar has shown increased survivorship at 22°C, advanced juveniles achieved highest survivorship at 25°C, and the on-farm grow-out phase in ponds has been recommended at around 27°C. The primary aim of this group of experiments was to quantify the effect of temperature on the oxygen consumption rate of each instar for redclaw *Cherax quadricarinatus* from egg through to J3 (the life stages considered for a hatchery and nursery phase) using closed-cell respirometry to produce a thermophysiological profile of each instar to compare and contrast instars, and to compare to the extant data. The suitable thermal regimes here were identified as; eggs from 22°C to 28°C, L1 from 22°C to 31°C, L2 from 20°C to 26°C, J1 from 20°C to 26°C, J2 from 22°C to 28°C, and J3 from 20°C to 26°C. By comparing to previously published survivorship data we believe that cooler temperature regimes are required for the early life stages up to J3 prior to release into on-growing ponds. Eggs are likely to survive best in the range 22°C to 25°C; the literature that exists for L1 shows maximum weight gain and moulting frequency at 28°C with maximum survival at 24°C - 30°C, so a combination of these factors suggests 25°C - 26°C would be suitable; no literature exists on L2, J1 and J3 except for J1, the recommendation would be for 22°C for all three of these instars; no literature exists for J2, however the recommendation would be the same as for eggs, and in both cases more towards 25°C. These lower nursery

phase temperatures are expected to increase survivorship during the grow-out stage, which would translate to higher stability in survivorship through to harvest.

5.2 Introduction

The freshwater crayfish *Cherax quadricarinatus*, (redclaw) is endemic to northern Australia including areas of the west of Queensland's Cape York Peninsula, easterly regions of the Northern Territory, and southern Papua New Guinea (Jones 1990; Jones and Ruscoe 2002; Webster *et al.* 2004; Bugnot and Lopez Greco 2009; Ghanawi and Saoud 2012; Saoud *et al*. 2013; Zhu *et al*. 2013; Stumpf *et al*. 2014). In these remote areas redclaw were well known as a large and highly valued wild-caught seafood, the subsequent introduction into commercial aquaculture was initiated in the late 1980's with high expectations and enthusiasm for development as a new aquaculture species (Jones 1990). Redclaw are advantageously easy to culture due to larval stages which occur under maternal care, a broad-ranging physiological tolerance to environmental conditions such as temperature, dissolved oxygen and salinity, suits translocation to tropical and sub-tropical areas globally, and a low-protein food requirement make them inexpensive to grow out to market size and achieve a premium price (Jones 1990; FAO 2017). Despite these many favourable attributes, the aquaculture industry for redclaw has failed to live up to expectations (Jones 1990; FAO 2017).

The main barrier to the development of a large-scale aquaculture industry for redclaw appears to be the variability at harvest, whereby there are high levels of unexplained mortality at times, which has induced uncertainty and inhibited investment. In other more established aquaculture industries, such as those for oysters and shrimp, the problem of variable harvest has been tackled through investment in

hatchery and nursery technology to produce good quality, robust seedstock (Paniagua-Chavez and Tiersch 2001; Arnold *et al*. 2013). The technology now exists to produce J1 instar redclaw seedstock in a dedicated hatchery for pond stocking for grow-out (Jones and Valverde 2020). The next step to tackling variable survivorship would be an additional nursery phase to take them through another two instars to J2 or J3 prior to release into grow-out ponds, and to develop a standard operating procedure to add robustness through the application of optimal feeding, temperature and habitat / shelter provisions. It is envisioned that the addition of a nursery phase to the production of seed stock in a hatchery will allow for farms to concentrate on grow-out, and that the enhanced robustness of the seed stock will translate into higher yields and less variability of survival through to harvest.

Within a hatchery phase and subsequent nursery phase, it is important to determine and apply the most suitable water temperature to each of the early instars. The consequence of environmental temperature is that it is an integral component of the physiological capacity of an organism to consume and convert food resources into growth, reproduction and survival (Sokolova *et al*. 2012). This is particularly important for organisms which cannot control their internal body temperature and are forced to match their environmental temperature (Jones and Shanks 2009). Thermoconformers in the marine or freshwater environments, such as redclaw, rely heavily on environmental thermal regimes for thermoregulation, therefore the culture temperature is of utmost importance (Jones and Shanks 2009).

The effects of temperature on survivorship and growth for redclaw from eggs to the third hatched instar (J1) has been extensively researched (Jones 1990; King 1994; Jones 1995c, b; Yeh and Rouse 1995; Anson and Rouse 1996; Barki *et al.* 1997b; Zhao *et al.* 2000; Cortés-Jacinto *et al.* 2003; García-Guerrero *et al.* 2003a,b; Karplus *et al.*

2003; Campaña-Torres *et al*. 2005, 2008; De Bock and López Greco 2009; Calvo *et al*. 2011; Calvo *et al.* 2013). One study examined the third hatched instar, J1, which would be involved in a nursery phase (Rigg *et al.* 2021a). The stages involved in grow-out through to adult harvest size have also been examined (Anson and Rouse 1996; Barki *et al*. 1997b; Cortés-Jacinto *et al*. 2003; Campaña-Torres *et al*. 2005, 2008; Calvo *et al.* 2011; Calvo *et al*. 2013). A sequential study on the effects of temperature for the full range of instars involved in hatchery and nursery phases following from eggs through to J3 has not been conducted.

There is evidence shown by temperature studies on redclaw that there are different presumed suitable thermal ranges for different instars. An optimum temperature striking a balance between incubation duration and hatch rate of eggs has been suggested as 22°C – 25°C for redclaw (Garcia-Guerrero *et al.* 2003a); early instar survivorship / growth experiments have been conducted at various temperatures (Rigg *et al.* 2021a), and the results suggest 22°C as the rearing temperature for increased survivorship for J1 instar; advanced juveniles (0.75 g +/- 0.23 g) achieved highest survivorship at 25°C but greatest biomass at 28°C (García-Guerrero *et al.* 2013); and the on-farm grow-out phase in ponds has been recommended at around 27°C (Jones 1995c). Thus, there appears to be evidence that there may be different thermal optima or preferred thermal ranges for the different life stages, particularly when looking at the interaction between survival and growth (García-Guerrero *et al.* 2013; Jones 1995c). This information is of particular importance in developing a standard operating procedure for hatchery and nursery phases for redclaw during the first six instars. One method of exploring presumed suitable thermal ranges is to measure oxygen consumption rates at different temperatures.

Metabolic rate is often quantified indirectly via respirometry, where the rate of oxygen consumption from 100% air saturation to a lower figure is ascertained. By measuring oxygen consumption over a range of temperatures it becomes possible to compare the suitability of different temperatures. Metabolic physiology studies allow for an understanding of the dynamics of energy use, losses, gains and efficiencies for cultured organisms (Brett and Groves 1979; Fitzgibbon 2010) and can be useful to study the effects of intrinsic influences such as physiological status or health (Dalosto and Santos 2011), phylogenetic differences, ontogeny, nutrition, and extrinsic elements such as temperature in aquatic environments (Anger 2001; Fitzgibbon 2010). Previous published research has quantified the effect of a variety of parameters on oxygen consumption in crustaceans in a number of ways, which include; determining differences and similarities within and between species (Bridges and Brand 1980), geographic locations (Dalosto and Santos 2011), diets (Swiss and Johnson 1976), anoxia (Hervant *et al.* 1998), activity (Torres and Childress 1983), aerobic scope (Ern *et al*. 2015) temperatures (Daoud *et al*. 2007), and life stages (Lemos and Phan 2001), amongst others. One area of thermophysiological research that has not been determined for *C. quadricarinatus* is the relationship between temperature and oxygen consumption at each instar between egg and J3.

To advance the aquaculture of redclaw as an industry a series of experiments is required to determine the suitable thermal regimes for each life stage. Due to the evidence that each life stage between egg and J3 may require different environmental temperature regimes, a series of experiments was conducted to determine a presumed suitable thermal range for each stage. The primary aim of this group of experiments was to quantify the effect of temperature on the oxygen consumption rate of each instar for redclaw *C. quadricarinatus* from egg through to J3 by producing a

thermophysiological profile of each instar to compare to each other to identify similarities and differences between the instars, and to compare the results to previously published temperature / survivorship data that exists for eggs, J1s and juveniles to recommend presumed suitable thermal ranges based on short-term data gathered here combined with finding a balance between growth and survivorship in long-term studies.

5.3 Materials and Methods

5.3.1 Experimental animals

Note: the naming convention used herein follows that published by Rigg *et al.* (2021b).

Cherax quadricarinatus were sourced from AquaVerde Redclaw Farm and Hatchery, 39 Blue Gum Rd, Carrington QLD 4883, 17.308230° S 145.459710° E. On-farm, advanced stage eggs were stripped from a broodstock female following the methods developed by Jones *et al.* (2018) and placed in baskets in the Hemputin incubator (Jones and Valverde 2020). The baskets were mechanically agitated with an oscillation rate of 15 times per minute, twice during the oscillation phase the baskets were mechanically lifted and dropped to rotate the eggs within the basket and prevent anoxia. The hatchery system was maintained at 26°C +/- 0.5°C and experimental animals were supplied at various life stages, either: eggs (late development stage eggs with visible eyes), L1 (larval stage 1, the first hatched instar, lecithotrophic, immobile), L2 (larval stage 2, post first moult, lecithotrophic, immobile), or J1 (first juvenile stage, second moult, exogenous-feeding, free-living).

All animals were held at 24°C for a minimum of two days prior to each experiment, to equilibrate all experimental animals to the same temperature. More advanced instars J2 (second juvenile stage, third moult, exogenous-feeding, free-living)

and J3 (third juvenile stage, fourth moult, exogenous-feeding, free-living) were produced by on-growing J1 redclaw in the laboratory facility at James Cook University in a recirculating system held at 24°C +/- 0.5°C in aged, oxygenated fresh water and provided with habitat to discourage cannibalism. Habitat consisted of bundles of dressmaker's Tulle. Instars J1, J2 and J3 were fed defrosted frozen *Artemia* sp. at the rate of 0.25 g L^{-1} day^{-1}, daily monitoring and recording was conducted for mortalities and moults. Non-exogenous feeding instars eggs, L1's and L2's were held in replicate apparatus without requirement for habitat or feed. Water quality was maintained through a water exchange of 20 L minimum per day per system (systems consisted of 3 experimental tubs [17 L each] plus a sump [15 L] [total 66 L]) which approximated to 30% water exchange each day. Water quality was maintained at: pH 7.2 – 7.6, Ammonia 0 - 0.25 ppm, Nitrite = 0, Nitrate 0 – 5 ppm. Exogenous-feeding animals from J1 to J3 were not fed for 24 hours prior to each experiment to account for possible variation in nutritional status or post-prandial metabolism.

5.3.2 Respirometry Equipment

The equipment used to measure oxygen consumption was the MicroResp™ version 1.0.4 Microplate System (Loligo Systems, Viborg, Denmark). Microrespirometry perspex plates were custom made, and light sensitive oxygen sensor reader spots (optodes) (Oxygen Sensor Spot SP-PSt5-) fixed to the centre of each chamber as per the manufacturer's instructions. Respiration rate chambers of 5.5 ml x 24 wells x 2 microplates (Appendix, Chapter 5.3.2. Plate 3) were used to accommodate *C. quadricarinatus* life stages from L1 to J3, chosen for their size to accommodate the experimental animals and to provide a large enough volume of air-saturated water for the period of the experiment. Chambers of 2.5 ml volume were chosen for experiments with eggs, appropriate for volume and air saturation in this instance, although due to

the high temperature and potentially high oxygen consumption, the 39°C temperature treatment for eggs was conducted in 5.5ml chambers. Each chamber included an oxygen optode (Appendix, Chapter 5.3.2. Plate 3) which conveyed data to the accompanying computer program via a 24-channel fluorescence-based respirometry system (SDR Sensor Dish Reader) (PreSens Precision Sensing GmbH, Regensburg, Germany) (Appendix, Chapter 5.3.2. Plate 4). Each plate held 20 experimental specimens in individual chambers and four controls with no specimens. The control chambers allowed for quantification of intrinsic factors such as background respiration, and / or extrinsic factors such as atmospheric pressure variation to be subtracted from each reading.

Micro respirometry plates were each placed in a 2 L container and filled with 1 L of vacuum-filtered (0.45 μm) aerated and aged (< 2 day) tap water with an integral airstone to maintain 100% air saturation, experimental animals were similarly placed in 1 L containers in the same fashion, these containers plus the weights used to hold the rubber covers in place for the microplate wells were placed in small water baths (13 L capacity, holding *c.* 8.5 L), connected to the main recirculating water bath system (52 L) to equilibrate all components to the same temperature. The component parts of the microplate system, the experimental animals and water to be used for the experiment were acclimated from 24°C to the experimental temperature, increased or decreased by 2°C per hour via a heater / chiller unit. Temperature change was conducted at 2°C per hour to allow for all experiments to be conducted during daylight hours to standardize measurements and exclude the potentially confounding effects of crepuscular activity or circadium rhythm fluctuations at night, which may affect oxygen use.

Experimental animals were individually loaded into the microplate wells without delay whilst still in the small water bath to maintain the correct experimental

temperature. The microplates were placed in the perspex temperature control bath housing, microbubbles were eliminated using a micropipette, the surface of the plate was flooded to produce a single meniscus across the entire surface, then covered with Parafilm®, followed by a rubber sealing pad and weight supplied for use with the system, as per the manufacturer's instructions. The perspex chamber housing the microplate had the lid secured and was flooded with water from the recirculating water bath, maintained at the experimental temperature.

Prior to the experimental trials, each plate was dual point calibrated for 0% O_2 and 100% O_2, following the manufacturer's instructions, at each temperature. 100% solution was obtained by vigorous aeration for approximately 1 h and 0% solution was obtained by the addition of 4% Na_2SO_3. The MicroResp^{TM} program was initiated and set to record data after the calibration changes had shown to have returned the readout traces to a stable reading. The experimental subjects in each instance were run at a minimum to the point at which all wells had reached 65% O_2 saturation or 2 hours of readings, which ever occurred first. The control wells were run simultaneously. Oxygen saturation within the wells was recorded every 60 seconds for the duration of the experiment. The first six instars of redclaw from egg to J3 were tested at ten temperatures from 16°C to 39°C, J3 instar were also trialed at 41°C, all with 20 replicates each.

At the conclusion of the experiments experimental animals were removed from the chambers, dried on absorbent paper for approximately three seconds and weighed, then placed on a 1mm graticule under a dissecting microscope and measured from the posterior of the eye cavity to the end of the cephalothorax in a straight line (ocular carapace length, [OCL], see Appendix Chapter 5.3.2. Plate 5). The redclaw were then placed in a -80°C freezer following which they were lyophilized and weighed to obtain

a lyophilized weight, a less-variable comparative for mass than wet weight for redclaw (Rigg *et al*. 2021b).

5.3.3 Data Analysis

Data from individual temperature experiments were transferred to Microsoft Excel (2013) and where necessary data trimmed to include the top 35% depletion of oxygen, i.e. 65% of the oxygen unused or 2 hours of readings, whichever occurred first. The control wells were averaged at each data point every 60 seconds and the mean value was subtracted from the raw data, after which a line was fitted, and regression performed to calculate the slope and associated error. Oxygen consumption rate was calculated as the slope for each replicate (oxygen consumption over time), for each temperature treatment, at each life stage and was presented as oxygen depletion per millimole of oxygen per hour (mmol h^{-1}) in each chamber. This figure was then divided by the lyophilized mass of the experimental subject to give mmol $g^{-1\ LM}$ h^{-1} as one of the major factors that contribute to oxygen consumption is mass, larger animals typically consume more oxygen (Crear and Forteath 2000).

Analyses were then conducted by transferring data to IBM SPSS Statistics 24 and compared via One-Way Analysis of Variance for independent variable oxygen consumption. *Post hoc* LSD analyses were conducted to establish significant differences between the temperature treatments at each stage and data plotted for the ten temperature treatments for each of the six life stages with error bars showing 95% confidence interval of the mean.

Each interval between temperatures was calculated via General Linear Model Univariate, analysis of covariance (ANCOVA), and compared to the following interval to establish where significant differences ($p < 0.05$) occurred. These differences were used to ascertain regions of non-significantly different slopes between temperatures for

each life stage. The start of these non-significantly different regions of the oxygen consumption plot was a change point after which the slopes of the lines between temperatures did not differ significantly up until a second change point was reached, where the oxygen consumption rate for the slope between temperatures were significantly different to that of the preceding oxygen consumption rate. This procedure was designed to identify a section of the data with a set of temperature ranges that were not significantly different from each other. Once the band of non-significantly different intervals was established, each individual interval was compared to the first interval via ANCOVA, any intervals which differed significantly from the first were discarded from the band.

5.4 Results

Temperature had a statistically significant effect on oxygen consumption for each life stage (statistics reported in Figures 18 – 24). The mean oxygen consumption rate for each instar across the range of temperatures showed bands where the oxygen consumption rate at temperatures and the intervening slopes did not differ significantly within the group (Figures 18 – 24; for the full analysis results see Appendix Chapter 5.4, Tables 7 and 8). The slopes for eggs illustrate a section of temperatures from 22°C - 28°C not differing significantly from each other (Figure 18), L1 instar redclaw showed a section from 22°C - 31°C where the slope of O_2 consumption was not significantly different (Figure 19), the slopes for L2 instar redclaw from 20°C - 26°C are not significantly different from each other (Figure 20), J1 instar has slopes from 20°C - 26°C not significantly different from each other (Figure 21).

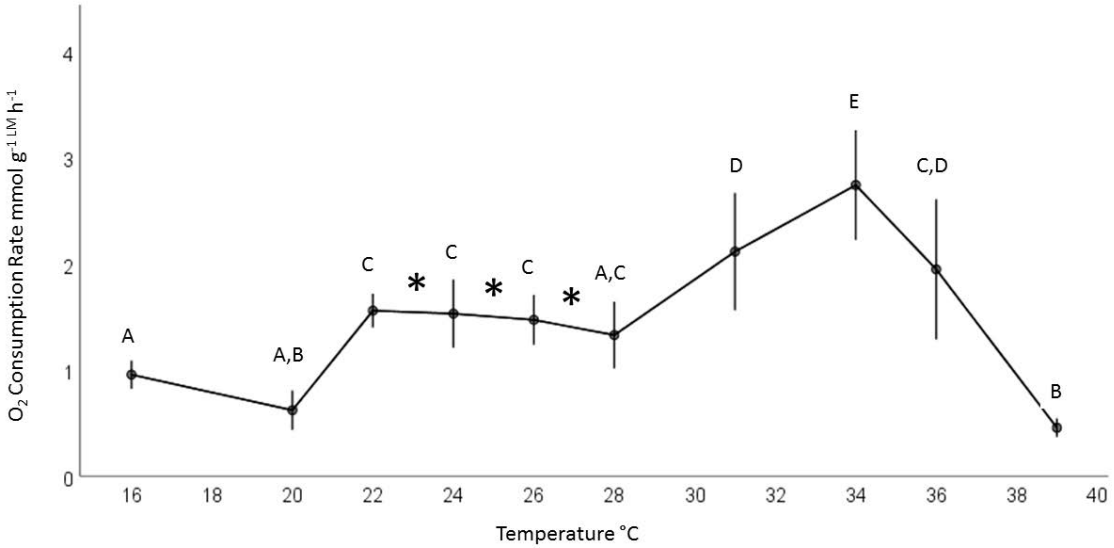

Figure 18. Oxygen consumption rate for *Cherax quadricarinatus* eggs across ten temperatures; temperature had a significant effect on O_2 consumption ($F_{9, 157} = 19.637$, $p < 0.001$).

Error bars with the same letter are not significantly different, the data was collected over a period varying between 15 minutes and 2 hours, depending on the temperature. Comparison of slopes for the intervals 22°C to 24°C and 24°C to 26°C were not significantly different ($F_{1, 65} = 0.016$, $p = 0.900$), comparison of slopes for 24°C to 26°C and 26°C to 28°C were not significantly different ($F_{1, 57} = 0.104$, $p = 0.748$), slopes with asterisks indicate the band of non-significantly different slopes, error bars indicate the 95% confidence interval. A comparative study by Garcia-Guerrero *et al.* (2003) recommended a best egg incubation temperature of 22°C - 25°C, with 25°C as being best for survival, energy cost and protein consumption.

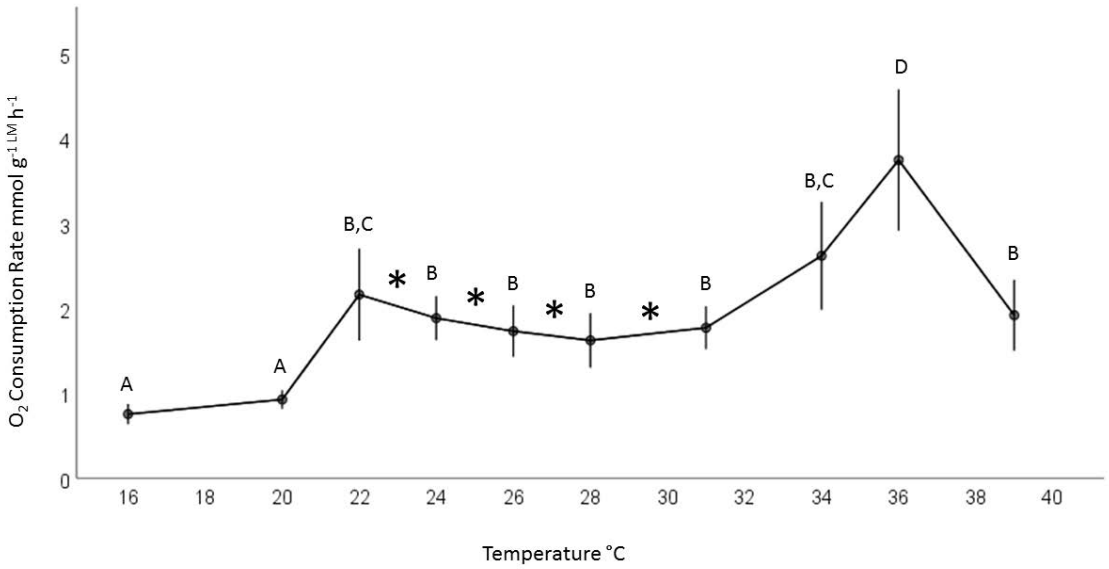

Figure 19. Oxygen consumption rate for L1 instar *Cherax quadricarinatus* across ten temperatures; temperature had a significant effect on O_2 consumption ($F_{9, 162} = 17.020$, $p < 0.001$).

Error bars with the same letter are not significantly different, the data was collected over a period varying between 15 minutes and 2 hours, depending on the temperature. Comparison of slopes for the intervals 22°C to 24°C and 24°C to 26°C were not significantly different ($F_{1, 71} = 0.138$, $p = 0.711$), comparison of slopes 24°C to 26°C and 26°C to 28°C were not significantly different ($F_{1, 69} = 0.025$, $p = 0.874$), comparison of the slopes 26°C to 28°C and 28°C to 31°C were not significantly different, ($F_{1, 63} = 0.819$, $p = 0.369$), slopes with asterisks indicate the band of non-significantly different slopes, error bars indicate the 95% confidence interval. A comparative study by Meade *et al.* 2002 for "newly hatched" redclaw which ran for 70 days found maximum weight gain and moulting frequency at 28°C, with maximum survival from 24°C - 30°C.

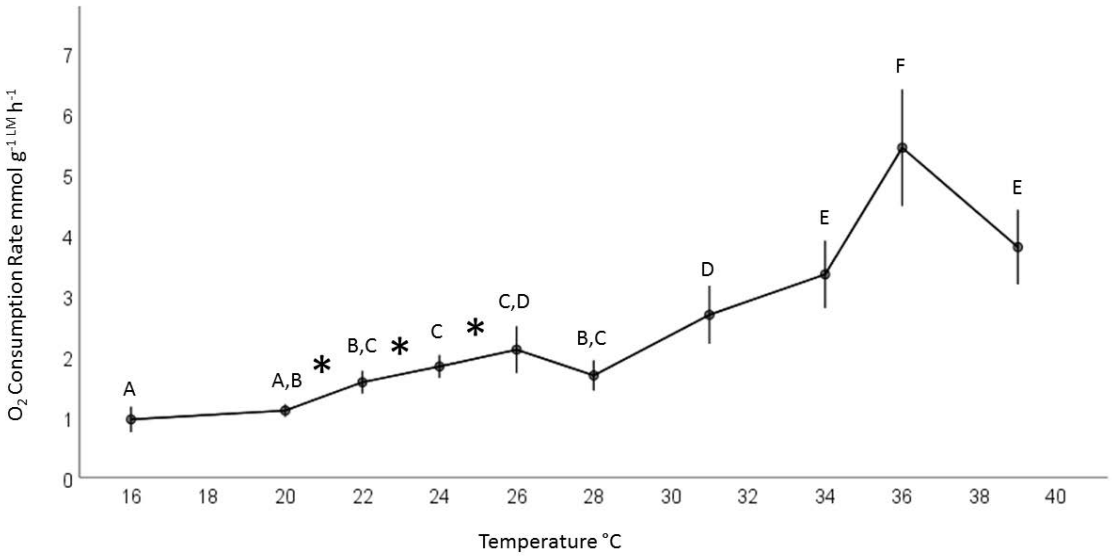

Figure 20. Oxygen consumption rate for L2 instar *Cherax quadricarinatus* across ten temperatures; temperature had a significant effect on O_2 consumption ($F_{9, 158} = 41.709$, $p < 0.001$).

Error bars with the same letter are not significantly different, the data was collected over a period varying between 15 minutes and 2 hours, depending on the temperature. Comparison of slopes for the intervals 20°C to 22°C and 22°C to 24°C were not significantly different ($F_{1, 68} = 1.738$, $p = 0.192$), comparison of the slopes 22°C to 24°C and 24°C to 26°C were not significantly different ($F_{1, 61} = 0.006$, $p = 0.941$), slopes with asterisks indicate the band of non-significantly different slopes, error bars indicate the 95% confidence interval. A comparative study by Meade *et al.* 2002 for "newly hatched" redclaw which ran for 70 days found maximum weight gain and moulting frequency at 28°C, with maximum survival from 24°C - 30°C.

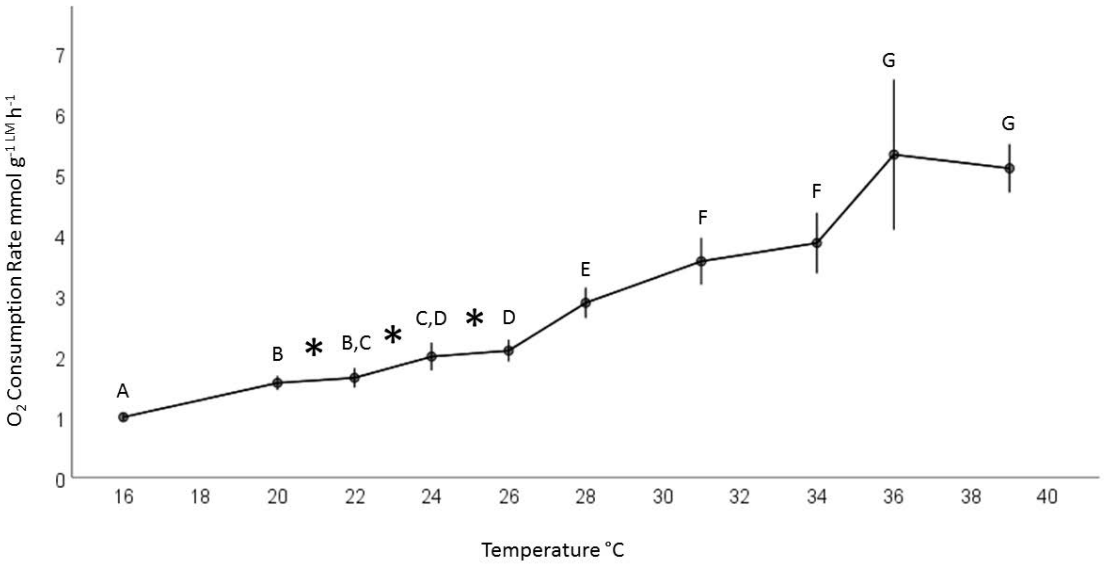

Figure 21. Oxygen consumption rate for J1 instar *Cherax quadricarinatus* across ten temperatures; temperature had a significant effect on O_2 consumption ($F_{9, 159} = 89.428$, $p < 0.001$).
Error bars with the same letter are not significantly different, the data was collected over a period varying between 15 minutes and 2 hours, depending on the temperature. Comparison of slopes for the intervals 20°C to 22°C and 22°C to 24°C were not significantly different ($F_{1, 71} = 2.827$, $p = 0.097$), comparison of the slopes 22°C to 24°C and 24°C to 26°C were not significantly different ($F_{1, 68} = 1.791$, $p = 0.185$), slopes with asterisks indicate the band of non-significantly different slopes, error bars indicate the 95% confidence interval. A comparative study by Rigg *et al.* (2021a) for redclaw starting at J1 which ran for 22 days, showed both the highest weight gain and the highest survivorship at 22°C.

The data for J2 instar redclaw shows a band of O_2 consumption slopes that do not differ statistically from each other from 22°C - 28°C (Figure 22), the results for J3 instar indicate a section of the data of non-significantly different O_2 consumption slopes from 20°C - 26°C (Figure 23).

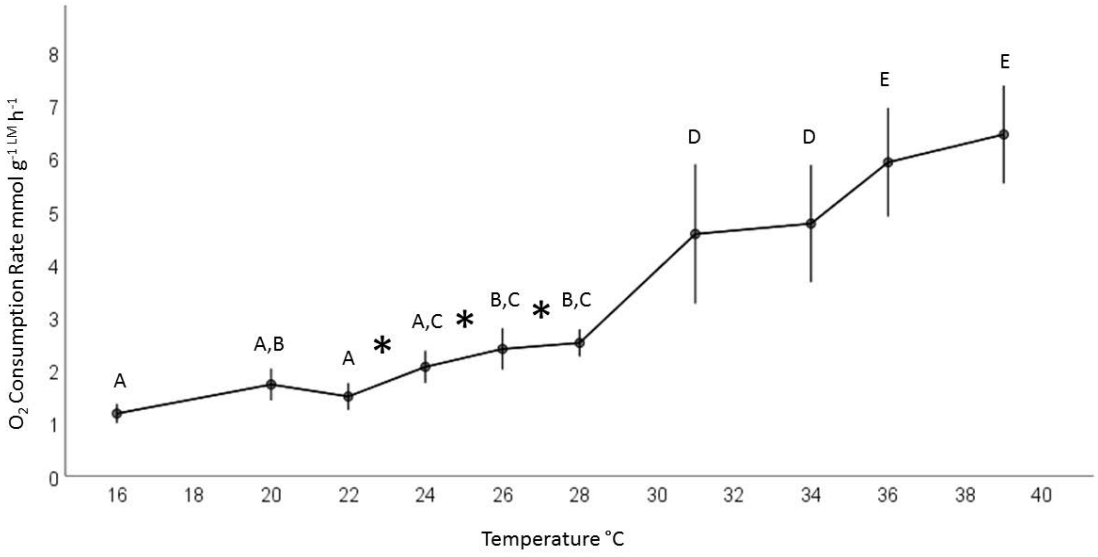

Figure 22. Oxygen consumption rate for J2 instar *Cherax quadricarinatus* across ten temperatures; temperature had a significant effect on O_2 consumption ($F_{9, 161} = 31.816$, $p < 0.001$).

Error bars with the same letter are not significantly different, the data was collected over a period varying between 15 minutes and 2 hours, depending on the temperature. Comparison of slopes for the intervals 22°C to 24°C and 24°C to 26°C were not significantly different ($F_{1, 72} = 0.523$, $p = 0.472$), comparison of the slopes 24°C to 26°C and 26°C to 28°C were not significantly different ($F_{1, 75} = 0.458$, $p = 0.501$), slopes with asterisks indicate the band of non-significantly different slopes, error bars indicate the 95% confidence interval. A comparative study by Rigg *et al.* (2021a) for redclaw starting at J1 which ran for 22 days, showed both the highest weight gain and the highest survivorship at 22°C.

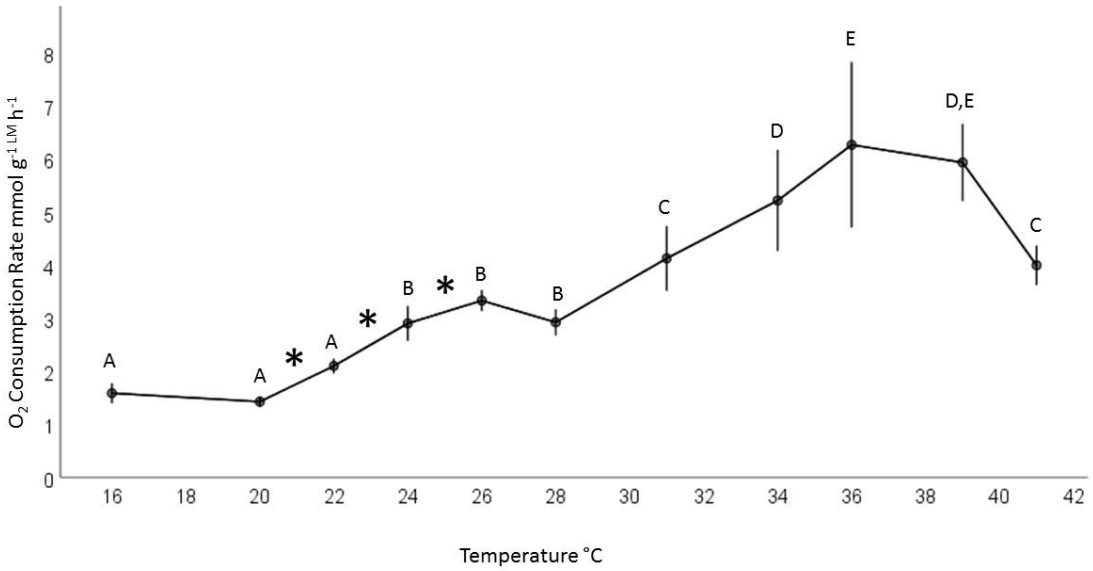

Figure 23. Oxygen consumption rate for J3 instar *Cherax quadricarinatus* across eleven temperatures; temperature had a significant effect on O_2 consumption ($F_{10, 166} = 39.794$, $p < 0.001$).
Error bars with the same letter are not significantly different, the data was collected over a period varying between 15 minutes and 2 hours, depending on the temperature. Comparison of slopes for the intervals 20°C to 22°C and 22°C to 24°C were not significantly different ($F_{1, 63} = 0.388$, $p = 0.536$), comparison of the slopes 22°C to 24°C and 24°C to 26°C were not significantly different ($F_{1, 71} = 1.999$, $p = 0.162$), slopes with asterisks indicate the band of non-significantly different slopes, error bars indicate the 95% confidence interval. A comparative study by Rigg *et al.* (2021a) for redclaw starting at J1 which ran for 22 days, showed both the highest weight gain and the highest survivorship at 22°C.

When the change points for the start and end of the first band of the non-significantly different temperature slopes were plotted for each instar based on comparative slope analysis identification, a pattern emerged where there was an increase in presumed suitable thermal range from egg (6°C range) to L1 (9°C range), with higher upper change points for L1 (31°C) as opposed to 28°C for eggs (Figure 24). From L2 to J3 the presumed suitable thermal range is 6°C but with a starting point lower than Egg and L1 of 20°C for L2, J1 and J3. J2 instar shifts 2°C higher in temperature range for lower and upper points than L2, J1 and J3 and matches the range for Eggs (Figure 24).

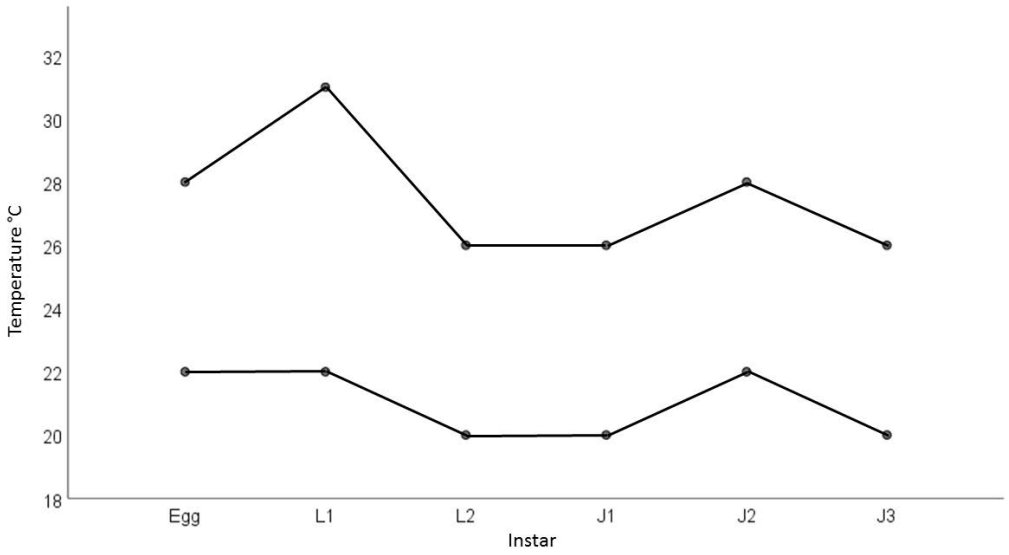

Figure 24. Short-term temperature ranges for six instars of *Cherax quadricarinatus* based on comparative slope analysis where the slope between temperatures did not vary significantly ($\alpha < 0.05$).

5.5 Discussion

This study forms the first in-depth exploration of presumed suitable thermal ranges for the first six instars of *C. quadricarinatus* from late stage egg through to instar J3, two further moults from the first independent, free-living, motile, exogenous-feeding instar J1, the instar that most redclaw would reach after a nursery phase lasting two to three weeks from J1 instar. This work also forms an important link between other studies which explored survivorship and growth over longer periods of time (Anson and Rouse 1996; Barki *et al.* 1997a; Meade *et al.* 2002; Cortés-Jacinto *et al*. 2003; Campaña-Torres *et al*. 2005, 2008; Calvo *et al*. 2011; Calvo *et al.* 2013; García-Guerrero *et al*. 2013, Rigg *et al.* 2021a).

Based on the data presented here, some presumed suitable thermal ranges can be construed. Furthermore, there is evidence of high thermal plasticity in the short term from the broad range of temperatures with statistically similar oxygen consumption

rates. It appears that cooler culture temperatures are required for the early instars, and each instar has a unique temperature requirement.

High temperatures often promote low survivorship over longer periods of time, and also may promote poor rates of growth as all available energy from food may be used to maintain high metabolic rate (Sokolova *et al.* 2012). As such, high temperatures, although survivable in the short term, may likely be fatal over a longer period (Sokolova *et al.* 2012). Here we have identified a band of non-significantly different slopes, a section of the data which has similar temperatures to the literature and one which other studies (Sammy 1988; Jones 1990; García-Guerrero *et al*. 2013; Rigg *et al*. 2021a) have shown provides good growth and survivorship over longer time periods.

The instars from egg to L2 are lecithotrophic non-exogenous feeding stages, relying on stored egg yolk for energy. A lower temperature for egg and L2 than that presently used in the AquaVerde hatchery which supplied animals for these experiments (26°C +/- 0.5°C), may be advantageous as the use of the stored yolk to fuel fast growth may leave the animals energetically unable to complete moults, which may be fatal (Song et al. 2017). High temperatures result in higher metabolism which may enable fast growth (Garcia-Guerrero *et al.* 2003a,b; Sokolova *et al.* 2012), but when associated with ecdysis, which is itself extremely energy-expensive (Bowser and Rosemark 1981; Villarreal 1991) and physiologically stressful (Saurabh and Sahoo 2008), higher metabolism with inadequate metabolic reserves may result in incomplete ecdysis where the animal perishes during the process (Bowser and Rosemark 1981; Saurabh and Sahoo 2008). The solution would be to either keep the temperature lower to enable the metabolic energy reserves of the stored yolk to meet the demands of ecdysis, or to enhance the stored energy of the egg yolk through enhanced nutrition of the broodstock or genetic selection for larger egg yolk reserves, or both. At present the

recommendation would be to keep the temperature lower until studies uncover routes to enhance the metabolic energy reserves of the egg yolk.

Combining information on thermal regimes from the literature with data generated here, some recommendations for culture temperature for each of the first six instars for redclaw can be established. The long-term recommendation regarding culture temperature for eggs from Garcia-Guerrero *et al.* (2003) is 22°C to 25°C, and here we show a short-term range of 22°C to 28°C (Figure 18). It is likely that in the long-term the temperature would be more suitable at a point lower than the top of the short-term range but at the top of the long-term range, therefore in this case the recommendation for culture temperature for eggs would be 25°C. Presently the only literature regarding L1 long-term temperature regimes is a study by Meade *et al.* (2002) for "newly hatched" redclaw which ran for 70 days and found maximum weight gain and moulting frequency at 28°C, with maximum survival from 24°C - 30°C; the short-term range identified here is 22°C to 31°C (Figure 19). Due to this broader range than that for eggs and higher upper temperature for the range, L1's may be better metabolically tolerant of a large and more variable temperature range. Instar L1 has the broadest short-term presumed suitable thermal range in this study of 9 degrees (22°C to 31°C) (Figure 19), which is interesting because there is quite a large change from the presumed suitable thermal range for egg instar which has a range of 6 degrees from 22°C to 28°C (Figure 18), and L2 instar which has a range of 6 degrees from 20°C to 26°C (Figure 20). This change in presumed suitable thermal range reflects an ontogenetic change which may have a behavioural component, allowing the maternal female with attached L1's to search more disparate microhabitats with varying temperatures for food, after carrying the eggs for around 30 days (Garcia-Guerrero *et al.* 2003a). The L1 instar animals are attached by dactylus hooks on their legs (Garcia-

Guerrero *et al.* 2003a) to the female's pleopods (Loya Javellana *et al.* 1994) which may provide more secure attachment to the female than that provided by the setae on the margins of the pleopods to which the eggs attach (Jones 1990); hence when egg-carrying, the female may be more reluctant to move around too much and source food close to her, for fear of detaching her eggs. Escape behaviour utilising a tail flick has been observed to detach eggs as well (D. Rigg, personal observation, unpublished), which may also encourage more sedentary behaviour to avoid confrontations with predators. Therefore, the female may need to search broadly for food resources in potentially unfavourable and / or highly variable temperatures, which the broad tolerance range of the L1's allows. The implications for the redclaw aquaculture industry of the larger presumed suitable thermal range for L1 instar are that culture temperatures may be raised or lowered for this instar, speeding up or slowing down development time to offset the lower temperatures and longer development times for other instars e.g. egg and L2, or allow the hatchery operator to manipulate the development time of this instar to synchronise production, or timing of production to coincide with ideal pond temperatures for release to grow-out.

Currently, there is no information on a long-term thermal range for instar L2 available in the literature, however here the indicated range is 20°C to 26°C (Figure 20). The only other published study to examine temperature over a longer period of time (22 days), but for instar J1, found an optimal temperature of 22°C (Rigg *et al.* 2021a) with almost complete survival at 22°C, but almost zero survival at 25°C. Somewhere in between lies the inflection point but without further resolution in the data the recommendation in this case for a long-term thermal optimum would be for 22°C, and as the presumed suitable thermal ranges uncovered here show the same range for L2 and J1 (Figure 24) the long-term recommendation would be the same for both

L2 and J1. This information is valuable as it contrasts with the current practice whereby nurseries produce eggs, L1, L2 and J1 at 26°C +/- 0.5°C (Jones and Valverde 2020) and the industry recommendation for release into grow-out ponds is 27°C (Jones 1995c). Clearly the temperatures we have outlined in this study are significantly lower, if applied to industry they may well contribute to higher survivorship and potentially slower growth, but reach a larger size both at harvest and through the earlier instars, where this would confer fitness for further grow-out.

The timing of release of post-nursery phase redclaw into grow-out ponds has a thermal determinant which may effect their survivorship and growth. The data presented here indicates that J1 and J3 animals have a short-term optimum between 20°C and 26°C (Figures 21, 23 & 24), and a long-term optimum of 22°C (Rigg *et al.* 2021a). The presumed suitable thermal range for J2 instar shifts towards warmer temperatures with a short-term recommendation of 22°C to 28°C (Figure 22), and closer to 25°C for the longer term. This has three implications, firstly, due to the fact that broodstock, hatchery and nursery phase can be controlled and performed at any time of the year, production of seedstock can be arranged to coincide with lower temperature conditions in the ponds at the start of Summer, ensuring a more suitable temperature environment and likely more survivorship. Secondly, strict mangement over broodstock, hatchery and nursery may be able to extend the grow-out season in length, or indeed enable more batches for grow-out. Furthermore, close observation of the nursery phase instars may allow for seeding of ponds at the J2 stage if the pond temperatures are slightly higher than those recommneded for J1 and J3, freeing up nursery facilities more quickly and allowing both a longer grow-out for these animals as well as allowing another batch to move through the nursery and hatchery more quickly. Adding a nursery phase allows for more control over thermal regimes for these

critical life stages and shows positive benefits as to timing for better batch control of seedstock and potentially more batches or longer grow-out to produce larger, more valuable redclaw at harvest.

Given the information gathered here regarding temperature regimes for each instar involved in a hatchery and a nursery phase, the addition of a nursery phase confers a number of benefits to the redclaw industry. As the timing of breeding for hatcheries can be artificially controlled, as is common practice, and that the instars from egg to J1 are already produced in a hatchery, the addition of a nursery phase has the potential to maximise survivorship and growth through the control of temperature regime, and also allow for accurate identification of the life stage. Furthermore, this can allow for release into grow-out ponds when temperatures are suitable earlier in the season, allowing for a longer growing season, or the possibility of more batches per annum. As the two major goals of growing redclaw are survivorship and growth through to harvest, a change in thermal regime to reflect the data for each instar, and at a lower temperature for egg and L2 instars may be of benefit in the long term. Future research should be directed to provide long-term data for each of the instars individually in the form of a growth and survivorship study, in order to ground-truth the results presented here.

5.6 Literature Cited

ANGER K (2001). The biology of decapod crustacean larvae. AA Balkema Publishers Lisse.

ANSON KJ AND ROUSE DB (1996). Evaluation of several commercial feeds and Crustacean Reference Diet for juvenile Australian Red Claw crayfish, *Cherax quadricarinatus*. *Journal of Applied Aquaculture* 6(1):65-76. doi: 10.1300/J028v06n01_06.

ARNOLD SJ, COMAN GJ AND EMERENCIANO M (2013). Constraints on seedstock production in eighth generation domesticated *Penaeus monodon* broodstock. *Aquaculture* 410:95-100. doi: 10.1016/j.aquaculture.2013.06.023.

BARKI A, LEVI T, HULATA G AND KARPLUS I (1997a). Annual cycle of spawning and molting in the red-claw crayfish, *Cherax quadricarinatus*, under laboratory conditions. *Aquaculture* 157(3-4):237-247. doi: 10.1016/S0044-8486(97)00163-4.

BARKI A, LEVI T, SHREM A AND KARPLUS I (1997b). Ration and spatial distribution of feed affect survival, growth, and competition in juvenile red-claw crayfish, *Cherax quadricarinatus*, reared in the laboratory. *Aquaculture* 148(2):169-177. doi: 10.1016/S0044-8486(96)01418-4.

BOWSER PR AND ROSEMARK R(1981). Mortalities of cultured lobsters, *Homarus*, associated with a molt death syndrome. Aquaculture 23(1-4):11-18.

BRETT J AND GROVES T (1979). Physiological energetics. *Fish physiology* 8(6):280-352.

BRIDGES C AND BRAND A (1980). Oxygen consumption and oxygen-independence in marine crustaceans. *Mar. Ecol. Prog. Ser* 2:133-141.

BUGNOT AB AND LOPEZ GRECO LS (2009). Structural alterations in the male reproductive system of the freshwater crayfish, *Cherax quadricarinatus* (Decapoda, Parastacidae). *J Invertebr Pathol* 102(2):160-166. doi: 10.1016/j.jip.2009.08.004.

CALVO NS, STUMPF L, PIETROKOVSKY S AND LÓPEZ GRECO LS (2011). Early and late effects of feed restriction on survival, growth and hepatopancreas structure in juveniles of the red claw crayfish *Cherax quadricarinatus*. *Aquaculture* 319(3-4):355-362. doi: 10.1016/j.aquaculture.2011.06.033.

CALVO NS, STUMPF L, SACRISTÁN HJ AND LÓPEZ GRECO LS (2013). Energetic reserves and digestive enzyme activities in juveniles of the red claw crayfish *Cherax quadricarinatus* nearby the point-of-no-return. *Aquaculture* 416-417:85-91. doi: 10.1016/j.aquaculture.2013.08.017.

CAMPAÑA-TORRES A, MARTINEZ-CORDOVA LR, VILLARREAL-COLMENARES H AND CIVERA-CERECEDO R (2005). In vivo dry matter and protein digestibility of three plant-derived and four animal-derived feedstuffs and diets for juvenile Australian redclaw, *Cherax quadricarinatus*. *Aquaculture* 250(3-4):748-754. doi: 10.1016/j.aquaculture.2005.02.058.

CAMPAÑA-TORRES A, MARTINEZ-CORDOVA LR, VILLARREAL-COLMENARES H AND CIVERA-CERECEDO R (2008). Carbohydrate and lipid digestibility of animal and vegetal ingredients and diets for the pre-adult redclaw crayfish, *Cherax quadricarinatus* (von Martens). *Aquaculture Research* 39(10):1115-1121. doi: 10.1111/j.1365-2109.2008.01980.x.

CORTÉS-JACINTO E, VILLARREAL-COLMENARES H, CIVERA-CERECEDO R AND MARTÍNEZ-CÓRDOVA R (2003). Effect of dietary protein level on growth and survival of juvenile freshwater crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae). *Aquaculture Nutrition* 9(4):207-213. doi: 10.1046/j.1365-2095.2003.00241.x.

CREAR B AND FORTEATH G (2000). The effect of extrinsic and intrinsic factors on oxygen consumption by the southern rock lobster, *Jasus edwardsii*. *Journal of Experimental Marine Biology and Ecology* 252(1):129-147. doi: https://doi.org/10.1016/S0022-0981(00)00243-4.

DALOSTO M AND SANTOS S (2011). Differences in oxygen consumption and diel activity as adaptations related to microhabitat in Neotropical freshwater decapods (Crustacea). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 160(4):461-466.

DAOUD D, CHABOT D, AUDET C AND LAMBERT Y (2007). Temperature induced variation in oxygen consumption of juvenile and adult stages of the northern shrimp, *Pandalus borealis*. *Journal of Experimental Marine Biology and Ecology* 347(1-2):30-40.

DE BOCK MS AND LÓPEZ GRECO LS (2009). Sex reversal and growth performance in juvenile females of the freshwater crayfish *Cherax quadricarinatus* (Parastacidae): effect of increasing temperature and androgenic gland extract in the diet. *Aquaculture International* 18(2):231-243. doi: 10.1007/s10499-008-9239-x.

ERN R, PHUONG NT, MADSEN PT, WANG T AND BAYLEY M (2015). Some like it hot: thermal tolerance and oxygen supply capacity in two eurythermal crustaceans. *Scientific reports* 5:10743. doi: https://doi.org/10.1038/srep10743.

FAO (2017). Cultured Aquatic Species Information Programme. *Cherax quadricarinatus*. Text by Jones, C. In: *FAO Fisheries Division* [online]. Rome. http://www.fao.org/fishery/culturedspecies/Cherax_quadricarinatus/en [accessed 21 January 2021].

FITZGIBBON Q (2010). Standard metabolic rate of spiny lobster (*Sagmariasus verreauxi*) pueruli determined by intermittent flow-through respirometry. *Journal of the Marine Biological Association of India* 52:264-273.

GARCIA-GUERRERO M, HENDRICKX ME AND VILLARREAL H (2003a). Description of the embryonic development of *Cherax quadricarinatus* (Von Martens, 1868) (Decapoda, Parastacidae), based on the staging method. *Crustaceana* V76 (3):269-280. doi: 10.1163/156854003765911676.

GARCIA-GUERRERO, M., VILLARREAL, H., & RACOTTA, I. S. (2003b). Effect of temperature on lipids, proteins, and carbohydrates levels during development from egg extrusion to

juvenile stage of Cherax quadricarinatus (Decapoda: Parastacidae). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *135*(1), 147-154. doi: 10.1016/s1095-6433(02)00354-9.

GARCÍA-GUERRERO M, HERNÁNDEZ-SANDOVAL P, ORDUÑA-ROJAS J AND CORTÉS-JACINTO E (2013). Effect of temperature on weight increase, survival, and thermal preference of juvenile redclaw crayfish *Cherax quadricarinatus*. *Hidrobiológica* 23(1):73-81.

GHANAWI J AND SAOUD IP (2012). Molting, reproductive biology, and hatchery management of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868). *Aquaculture* 358-359:183-195. doi: 10.1016/j.aquaculture.2012.06.019.

HERVANT F, MATHIEU J AND MESSANA G (1998). Oxygen consumption and ventilation in declining oxygen tension and posthypoxic recovery in epigean and hypogean crustaceans. *Journal of Crustacean Biology* 18(4):717-727.

JONES CM (1995b). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens)(Decapoda, Parastacidae) I. Development of hatchery and nursery procedures. *Aquaculture* 138(1995):221-238. doi: 10.1016/0044-8486(95)00068-2.

JONES CM (1995c). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens)(Decapoda, Parastacidae) II. Juvenile nutrition and habitat. *Aquaculture* 138(1995):239-245. doi: 10.1016/0044-8486(95)00069-0.

JONES C (1990). The biology and aquaculture potential of the tropical freshwater Crayfish *Cherax quadricarinatus*. *Queensland Department of Primary Industries Information Series* (qi-90028):I-XV, 1-109.

JONES C AND SHANKS S (2009). Requirements for the aquaculture of *Panulirus ornatus* in Australia. *Spiny lobster aquaculture in the Asia–Pacific region*:98.

JONES CM AND RUSCOE IM (2002). Biological and aquaculture characteristics of five stocks of redclaw, *Cherax quadricarinatus* (von Martens)(Decapoda: Parastacidae) from northern Queensland, Australia Freshwater Crayfish. Pp. 115-135. Proceedings of the Thirteenth Symposium of the International Association of Astacology.

JONES C, VALVERDE C AND RIGG D (2018). Redclaw Crayfish Hatchery. Development of Mass Production Hatchery for Cherax quadricarinatus. The Association of Astacology's Twenty-Second International Symposium on Freshwater Crayfish. International Association of Astacology, Carnegie Museum of Natural History, Pittsburgh, Pensylvania, USA.

JONES C.M. AND VALVERDE C (2020). Development of mass production hatchery technology for the redclaw crayfish, *Cherax quadricarinatus. Freshwater Crayfish*. 25, 1-6. doi: https://doi.org/10.5869/fc.2020.v25-1.001.KARPLUS I, GIDEON H AND BARKI A (2003). Shifting the natural spring–summer breeding season of the Australian freshwater crayfish *Cherax quadricarinatus* into the winter by environmental manipulations. *Aquaculture* 220(1-4):277-286. doi: 10.1016/s0044-8486(02)00225-9.

KARPLUS I, GIDEON H AND BARKI A (2003). Shifting the natural spring–summer breeding season of the Australian freshwater crayfish Cherax quadricarinatus into the winter by environmental manipulations. Aquaculture 220(1-4):277-286. doi: 10.1016/s0044-8486(02)00225-9.

KING CR (1994). Growth and survival of red claw crayfish hatchlings (*Cherax quadricarinatus* von Martens) in relation to temperature, with comments on the relative suitability of *Cherax quadricarinatus* and *Cherax destructor* for culture in Queensland. *Aquaculture* 122:75-80. doi: 10.1016/0044-8486(94)90335-2.

LEMOS D AND PHAN V (2001). Ontogenetic variation in metabolism, biochemical composition and energy content during the early life stages of *Farfantepenaeus paulensis* (Crustacea: Decapoda: Penaeidae). *Marine Biology* 138(5):985-997.

LOYA-JAVELLANA GN, FIELDER DR AND THORNE MJ (1994). Ontogeny of foregut in the tropical freshwater crayfish, Cherax quadricarinatus von Martens, 1868 (Parastacidae: Decapoda). Invertebrate reproduction & development 25(1):49-58.

MEADE ME, DOELLER JE, KRAUS D AND WATTS SA (2002). Effects of Temperature and Salinity on Weight Gain, Oxygen Consumption Rate, and Growth Efficiency in Juvenile Red-Claw Crayfish *Cherax quadricarinatus*. *Journal of the World Aquaculture Society* 33(2):188-198.

PANIAGUA-CHAVEZ C AND TIERSCH T (2001). Laboratory studies of cryopreservation of sperm and trochophore larvae of the eastern oyster. *Cryobiology* 43(3):211-223. doi: 10.1006/cryo.2001.2346.

RIGG DP, COURTNEY RL, SEYMOUR JE AND JONES CM (2021a). Determining Suitable Thermal Regimes for Early Instar Redclaw Juveniles, *Cherax quadricarinatus* (von Martens, 1868) (Decapoda, Parastacidae), for a Proposed Nursery Phase. Freshwater Crayfish 26(1): 1-7.

RIGG DP, COURTNEY RL, JONES CM AND SEYMOUR JE (2021b). Morphology and Weight-length Relationships for the First Six Instars of *Cherax quadricarinatus* (von Martens, 1868). Freshwater Crayfish 26(1): 1-8.

SAMMY N (1988). Breeding biology of *Cherax quadricarinatus* in the Northern Territory. Pp. 79-88 *In:* Evans LH and O'Sullivan D, eds.). Proceedings of the First Australian Aquaculture Conference, Perth, 23-27 October, 1988. Curtin University of Technology, Western Australia.

SAOUD IP, GHANAWI J, THOMPSON KR AND WEBSTER CD (2013). A review of the culture and diseases of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868). *Journal of the World Aquaculture Society* 44(1):1-29. doi: 10.1111/jwas.12011.

SAURABH S AND SAHOO P (2008). Major diseases and the defence mechanism in giant freshwater prawn, Macrobrachium rosenbergii (de Man). Proceedings of the National Academy of Sciences, India, Section B 78:103-121.

SOKOLOVA IM, FREDERICH M, BAGWE R, LANNIG G AND SUKHOTIN AA (2012). Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates. *Marine environmental research* 79:1-15. doi: https://doi.org/10.1016/j.marenvres.2012.04.003.

SONG Y, VILLENEUVE DL, TOYOTA K, IGUCHI T AND TOLLEFSEN KE (2017). Ecdysone Receptor Agonism Leading to Lethal Molting Disruption in Arthropods: Review and Adverse Outcome Pathway Development. Environmental science & technology 51(8):4142-4157. doi: 10.1021/acs.est.7b00480.

STUMPF L, TROPEA C AND LÓPEZ GRECO LS (2014). Recovery growth of *Cherax quadricarinatus* juveniles fed on two high-protein diets: Effect of daily feeding following a cyclic feeding period on growth, biochemical composition and activity of digestive enzymes. *Aquaculture* 433:404-410. doi: 10.1016/j.aquaculture.2014.06.037.

SWISS J AND JOHNSON M (1976). Energy dynamics of two benthic crustaceans in relation to diet. *Journal of the Fisheries Board of Canada* 33(11):2544-2550.

TORRES J AND CHILDRESS J (1983). Relationship of oxygen consumption to swimming speed in *Euphausia pacifica*. *Marine Biology* 74(1):79-86.

VILLARREAL H (1991). A partial energy budget for the Australian crayfish Cherax tenuimanus. Journal of the World Aquaculture Society 22(4):252-259. doi: 10.1111/j.1749-7345.1991.tb00742.x

WEBSTER CD, THOMPSON KR, MUZINIC LA, YANCEY DH, DASGUPTA S, XIONG YL, ROUSE DB AND MANOMAITIS L (2004). A preliminary assessment of growth, survival, yield, and economic return of Australian red claw crayfish, *Cherax quadricarinatus*, stocked at three densities in earthen ponds in a cool, temperate climate. *Journal of Applied Aquaculture* 15(3-4):37-50. doi: 10.1300/J028v15n03_03.

YEH HS AND ROUSE DB (1995). Effects of water temperature, density, and sex ratio on the spawning rate of red claw crayfish *Cherax quadricarinatus* (von Martens). *Journal of the World Aquaculture Society* 26(2):160-164. doi: 10.1111/j.1749-7345.1995.tb00239.x.

ZHAO Y, MENG F, CHEN L, GU Z, XU G AND LIU Q (2000). Effects of Different Gradient Temperatures on Embryonic Development of the *Cherax quadricarinatus* (Crustacea, Decapoda). *Journal of Lake Sciences* 12(1):59-62.

ZHU H, JIANG Q, WANG Q, YANG J, DONG S AND YANG J (2013). Effect of dietary carbohydrate-to-lipid ratios on growth performance, body composition, hepatic enzyme activities, and digestive enzyme activities of juvenile Australian Redclaw Crayfish, *Cherax quadricarinatus* (von Martens). *Journal of the World Aquaculture Society* 44(2):173-186. doi: 10.1111/jwas.12024.

Chapter 6. Additional Pilot Studies and Investigations into Factors Pertaining to a Nursery Phase for Redclaw Crayfish *Cherax quadricarinatus.*

Statement of the Contribution of Others

Damian Rigg is the primary author of this chapter and was extensively involved in all aspects of this work under the supervision of: Associate Professor Clive Jones, Professor Jamie Seymour and Dr. Robert Courtney. Additionally, Robert Courtney was involved in experimental design and procedure for the Biofloc experiments. Jessica Sleeman, Sally Browning and numerous aquarium volunteers assisted with animal husbandry. Andrew Jeffs of the National Institute of Water and Atmospheric Research (New Zealand) determined protein and lipids levels for section 6.4. Financial support was supplied by AgriFutures Australia (formerly RIRDC) which provided all necessary equipment and funding for travel and conference attendance. The research was supported by the North Queensland Crayfish Farmer's Association and AquaVerde Redclaw Crayfish Farm and Hatchery which supplied experimental animals and broodstock (www.AquaVerde.com.au), grateful thanks to Colin and Ursula Valverde. The Australian Postgraduate Award (APA) scholarship provided financial support for Damian Rigg during this candidature.

6.1 Abstract

Introducing a nursery phase to the aquaculture for redclaw requires a best practice model which delivers high growth rates and survivorship. Three studies were initiated as pilot or unreplicated studies to potentially map future directions for a nursery phase. Biofloc technology seeks to promote natural production of bacteria, algae and protozoans, for food, shading, processing wastes, and disease mitigation. Biofloc was applied to a tank system to gauge the survivorship and growth of juvenile redclaw, and as a potential nursery approach in tanks. A protocol was developed to produce biofloc cultures, the starter culture was then used to evaluate growth and survival of juvenile redclaw as compared to a Clearwater treatment. Biofloc cultures were produced, with or without crayfish within 14 days, the use of crayfish shortened the time to produce the biofloc, and reduced ammonia down to zero at day 20. The redclaw in the biofloc treatment had 1.45 x larger end mean weight, 3 x larger total weight, 27% higher survival, 78% higher weight gain, and 87% higher biomass than a clearwater treatment. The animals derived nutritional benefit from the biofloc in terms of survivorship and growth, however significant cannibalism made it less clear where nutrition came from, the diet, biofloc or conspecifics. There was also some evidence for uptake of dissolved organic matter (DOM) via means other than the digestive tract.

A key challenge for redclaw aquaculture based on hatchery generated seedstock is the inconsistency of harvest production. Such variability may be attributable to variance in the quality of the seedstock. Although hatchery produced seed redclaw are sold to farmers at the J1 stage and a consistent size of *c.* 18 mg, their nutritional status and fitness for grow-out are unknown. A measure of condition indicating potential for growth and survival of the crayfish would be an excellent tool for the redclaw aquaculture industry. In lobster physiology studies the quantum of lipid in the body can

be used as a measure of condition. The lipid and protein composition of redclaw juveniles was measured similarly to assess the utility of these metrics as a proxy for physiological condition. Growth and survivorship data were compared in terms of wet weight, dry weight and ocular carapace length, and analysed against diet treatments. The CSIRO diet treatment had the best survivorship for any of the diet treatments, albeit still extremely low, so perhaps the percentage proportion of protein of dry weight may be a good index for survival. The percentage lipids of dry weight is not a good index for condition of growth, nor is it a good index for survival either. The proportion of protein as percentage of dry weight of redclaw in the study in terms of wet weight, dry weight and ocular carapace length shows that percentage protein of dry weight is also not a good index for condition of growth.

A nursery phase in tanks requires maximum stocking density to lower per unit costs, thus cannibalism needs to be mitigated to increase stocking density and survivorship. One method to mitigate cannibalism is to provide habitat or structure, this study trialed two types and two sizes of scaled-down habitat, bowtie mesh arrangements constructed from dressmaker's tulle and tube habitat constructed from drinking straws. The bowtie microhabitat provided a habitat which offered less protection of the redclaw from each other than the tube microhabitat as the survival was much lower. Crayfish were able to see each other through the bowtie mesh and potentially cannibalise others, though the mesh of the bowtie microhabitats collected the food close to the animals, hence the large weight gain. By contrast, the tube microhabitat treatments offered significantly higher survival percentage but required the animals to leave the protection of the tubes to feed which may have discouraged them to feed hence the lower weight gain. The density treatments of 11-52 redclaw per m^2 surprisingly had no significant effect on either percentage weight gain or percentage survival though these densities

appeared to be quite high in view of the literature. The authors suspect habitat of one to two orders of magnitude larger is required, as the survivorship in this study was low across all treatments (8% large bowties – 37% long tubes). Mesh bowties would appear to be more practical as habitat, redclaw could easily out-grow tubes in an extended study, the mesh captures food items, is easy to clean and use, and practical in terms of harvesting. A broad range of stocking densities could be trialed alongside much larger microhabitat treatments to see if stocking density can be extended to its upper limits whilst negating large-scale conspecific predation.

6.2 Introduction

In the field of aquaculture many exciting new innovations and refinements of existing practices and protocols are occurring constantly. The aquaculture industry of redclaw crayfish is reasonably extensive in practise and just beginning to become more intensive, with the initiation of a hatchery phase, and the work presented in this Thesis, which contributes to the understanding of factors involved in a proposed nursery phase. Despite these advancements there are many more potential factors to explore which could influence the growth and survivorship of redclaw in a nursery phase, probably all of which are adaptations from other aquaculture industries.

6.2.1 Opportunity to Perform Additional Research on Factors Pertaining to a Nursery Phase for Redclaw Crayfish, *Cherax quadricarinatus*

The research presented in Chapters 2 to 5 of this thesis represent rigorous, replicated experiments designed to address the primary objectives of the RIRDC project that this PhD study was part of. During the course of the project however, additional research was performed of a preliminary or exploratory nature, the results of which are of scientific value and worthy of presentation here. This research provides information

of practical value to the redclaw farming industry which may form the basis for further investigations.

6.2.2 Purpose and Justification for Pilot Studies and Unreplicated Trials

The first of the additional research topics was the application of biofloc technology (Avnimelech 2009) to the culture of redclaw juveniles. Biofloc technology is an existing aquaculture approach that stimulates production of microorganisms in the water that serve to provide food, shading and waste uptake (Avnimelech 2009). This may have a particular advantage for redclaw in tank systems, as it provides an environment that is most similar to the natural environment that redclaw inhabits, one which features static water and high turbidity (Jones 1989). A preliminary trial to assess redclaw in a biofloc system was therefore strongly justified.

The second topic of additional research was a preliminary examination of the condition of redclaw juveniles as measured by the proximate composition of the body. The opportunity arose to have samples of redclaw from one of the replicated experiments analysed for their protein and lipid content to ascertain whether such measurements are good indicators of condition. Such data have been used for studies of condition in the puerulus stage of marine rock lobsters (Jeffs *et al.* 1999) – a life stage equivalent to the J1 stage of redclaw.

A preliminary experiment to examine density of juvenile redclaw in relation to provision of shelter was the third additional research topic. As tank based nursing of redclaw is in development, having never been applied by industry previously, the practical limits to density and the nature of artificial shelter are entirely speculative. Shelter for sub-adult to adult redclaw is used in grow-out ponds to allow for higher density (Jones and Ruscoe 2001), and to ameliorate cannibalism (Jones 1989), in tank-

based nursery phase experiments cannibalism has been a problem (Garza De Yta 2009), provision of shelter may provide a solution. To provide a baseline to the subsequent design of more rigorous, replicated experiments, an initial trial was performed to examine several densities (crayfish per two dimensional area) and putative shelter types.

6.3 Application of Biofloc to Redclaw Nursery Phase in Tanks

6.3.1 Introduction

Biofloc technology is an emerging technology applied to aquaculture in both tanks and more traditional outdoor ponds, for marine, brackish water and freshwater systems (El Sayed 2021). It seeks to promote the natural production of bacteria, algae and protozoans that become aggregated within a matrix of organic particulate matter – known as a floc (El Sayed 2021; Ulloa Walker *et al*. 2020). The biofloc provides a source of nutritious food, shading and processing of organic wastes, and is thought to have additional benefits to cultured species, such as disease mitigation (El Sayed 2021; Emerenciano *et al*. 2013; Shyne Anand *et al*. 2017; Li *et al*. 2019; Ulloa Walker *et al*. 2020).

In commercial aquaculture of redclaw, some farms have applied pond management regimes that inadvertently create biofloc formation. Mr Peter Moore owner/operator of CheraxPark, a redclaw farm located north of Gympie in southeast Queensland, applied the stocking of lucerne hay to his ponds (Peter Moore, personal communication circa 2016) which resulted in high turbidity water with apparent flocs of organic matter. He sought this to provide shading for the redclaw, known to prefer low light conditions (Jones 1990) and to create additional natural food for the redclaw.

This approach actually created a biofloc, which appeared to support improved growth and survival of the redclaw stock.

Managed biofloc systems involve the application of organic matter to the culture water within a regime that maintains a desired carbon to nitrogen ratio (C: N), typically in static water that is vigorously aerated (Ekasari *et al.* 2016). The organic matter input is a combination of the feed supplied to the cultured species and additional organic matter (e.g. molasses, yeast, bran) to achieve the desired C: N ratio and can be equally applied to a tank or pond system (Ekasari *et al.* 2016).

For this study, biofloc was applied to a tank system to gauge the response of juvenile redclaw in terms of survivorship and growth, and as a preliminary assessment of biofloc as a potential nursery approach in tanks. It was considered suitable because redclaw prefer dark conditions and high water turbidity in their natural environment (Jones 1990). Juvenile redclaw are known to consume the microorganisms and biofilms that the biofloc would provide (Viau *et al*. 2012), and biofloc may add advantages in terms of quality and quantity of feed with very little cost input. The aims in this study were to establish a repeatable method for producing biofloc and measure the water quality parameters to determine the capacity for nitrogen cycling and to quantify the effect of biofloc on redclaw crayfish in terms of growth and survival.

6.3.2 Methods and Materials

The pilot trial for the application of biofloc in small tanks was conducted in two parts, firstly a protocol was developed to produce biofloc cultures via adapted and combined methods as used by Ekasari *et al*. (2016) and Ballester *et al*. (2017) where regular additions of organic carbon in the form of molasses was used to stimulate the growth of heterotrophic microbial biofloc. Once the starter culture was established it

was then used in a further pilot study to evaluate the possible benefit to growth and survival of juvenile redclaw.

6.3.2.1 Starter Culture

The treatments chosen for the first part of this trial, biofloc starter cultures, were based on the recent literature by Ballester *et al.* (2017) and Ekasari *et al.* (2016), and two treatments were trialed, one with crayfish and one without crayfish. The intention was to test whether a biofloc culture could be produced in the first instance, and secondly what the effect of the addition of crayfish had on the production of biofloc. The Biofloc + Crayfish treatment received a doubling of molasses designed to offset the extra ammonia produced by the redclaw as the molasses served to "feed" the introduction of nitrobacter. By way of comparison the biofloc without crayfish culture had an equal mass of ammonia-producing pellets and molasses without crayfish. The experiment ran for 24 days.

Redclaw crayfish instar L1 were sourced from AquaVerde Redclaw Farm and Hatchery (17.3090°S, 145.4593°E, www.aquaverde.com.au) and placed in experimental systems at James Cook University. Each recirculating system consisted of two tanks (8 L each) and a 10 L sump, for a total system volume of 26 L, and a flow rate of 60 L H^{-1} per tank. Water was delivered via a spray bar in the water column to assist in water circulation. These animals were on-grown for 28 days to reach J2 instar, fed defrosted *Artemia* sp. *ad libitum* daily. Water temperature was maintained at air conditioned ambient room temperature *c.* 25°C, air stones in the sumps provided aeration of the water. Ammonia, nitrite, nitrate and pH readings were taken periodically throughout the experiment (days 3, 4, 6, 7, 11, 14, 17, 20 and 24) using an API Freshwater Master Test Kit. Crayfish were weighed for wet weight *en masse* to reach as close to 5 g as was possible (4.366 g).

The 15 L treatment with crayfish was started with 5 g CSIRO pellets, 10 g Molasses, and 4.366 g J2 instar redclaw. The 15 L treatment without crayfish was started with 5 g CSIRO pellets, and 5 g Molasses. Additional Molasses was added when total ammonia nitrogen values were taken, as per Ballester *et al.* (2017), calculated as follows.

1. Total Ammonia Nitrogen (ppm) × Volume (ml) = Total Quantity of Ammonia Nitrogen.

2. Total Quantity of Ammonia Nitrogen × 12 = Carbon Required to be added.

3. Molasses is the Carbon Source and Consists of 53% Carbon (Ekasari *et al.* 2016).

4. Therefore:

Molasses Required to be Added (g) = Carbon Required to be Added / 0.53.

At the completion of the experiment plotting of Total Ammonia Nitrogen, Ammonia, Nitrite and Nitrate across the period of the trial were conducted in IBM SPSS Statistics 24.

6.3.2.2 Biofloc versus Clearwater

Once the starter culture had been established, the second part of this trial was initiated. The culture without crayfish was used in preparing the biofloc treatment to compare to a clear water or non-biofloc treatment. The treatments were prepared as follows.

Redclaw crayfish were sourced from AquaVerde Redclaw Farm and Hatchery (17.3090°S, 145.4593°E, aquaverde.com.au) and on-grown and held in an identical fashion to those used in the starter culture (above) except that juvenile redclaw were fed defrosted frozen *Artemia* sp. at the rate of 10% of starting redclaw biomass per day. Ammonia, nitrite, nitrate and pH readings were taken periodically throughout the experiment (days 1, 4, 9, 14, 16, 18 and 21) using an API Freshwater Master Test Kit.

Crayfish (n= 150) for each treatment were weighed for wet weight *en masse* to reach as close to 4 g as was possible;

Biofloc total mass = 4.085 g, $\overline{x}$ = 0.0272 g,

Clearwater total mass = 4.067 g, $\overline{x}$ = 0.0271 g. The experiment ran for 21 days.

The 120 L Biofloc treatment consisted of 105 L of aged tap water plus a 15 L treatment without crayfish, 13.6 g Molasses, dressmaker's Tulle (habitat) 1500 × 800 mm with ceramic electric insulator at each end for negative buoyancy, airstones, and 150 × J2 instar redclaw crayfish total mass 4.085 g mean mass 0.0272 g.

The 120 L Clearwater treatment consisted of 120 L of aged tap water, plus dressmaker's Tulle (habitat) 1500 × 800 mm with ceramic electric insulator at each end for negative buoyancy, 150 × J2 instar redclaw crayfish total mass 4.067 g mean mass 0.0271 g. Water temperature was maintained at air conditioned ambient room temperature *c.* 25°C.

At the completion of the experiment plotting of mean start and end weights, total start and end weights, survival percentage, percentage weight change and percentage of original biomass were conducted in IBM SPSS Statistics 24.

6.3.3 Results

The starter culture treatments produced peak levels of total ammonia nitrogen three days apart but at the same level of 28.25 ppm for both treatments (Figure 26), ammonia levels peaked quickly at day five and remained high for both treatments until day 20 when the crayfish treatment returned to zero but non-crayfish treatment remained high (Figure 27). Nitrite levels peaked on the same day for both treatments, then reduced to the same rate (Figure 28), nitrate levels peaked three days apart for the two treatments and then reduced to the same level but also three days apart (Figure 29).

Floc was measured in settling cones at day 14 of the experiment and returned readings of: Biofloc with crayfish = 7 ml/ L, Biofloc without crayfish = 12 ml/L.

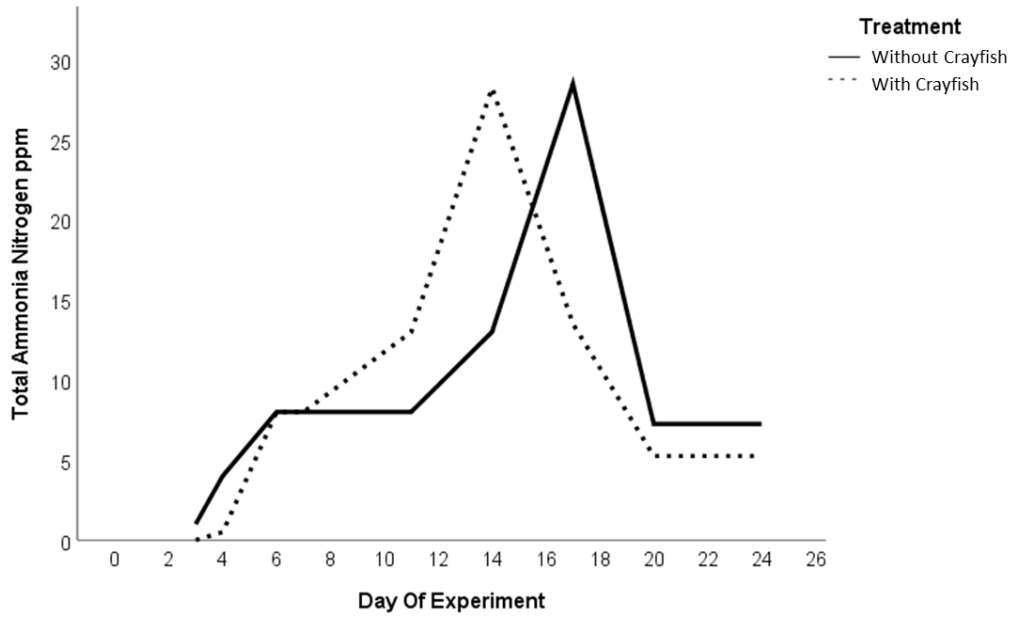

Figure 25. Total ammonia nitrogen per day of 24-day tank biofloc experiment for *Cherax quadricarinatus* nursery phase. Starter culture treatments were with redclaw crayfish and without redclaw crayfish.

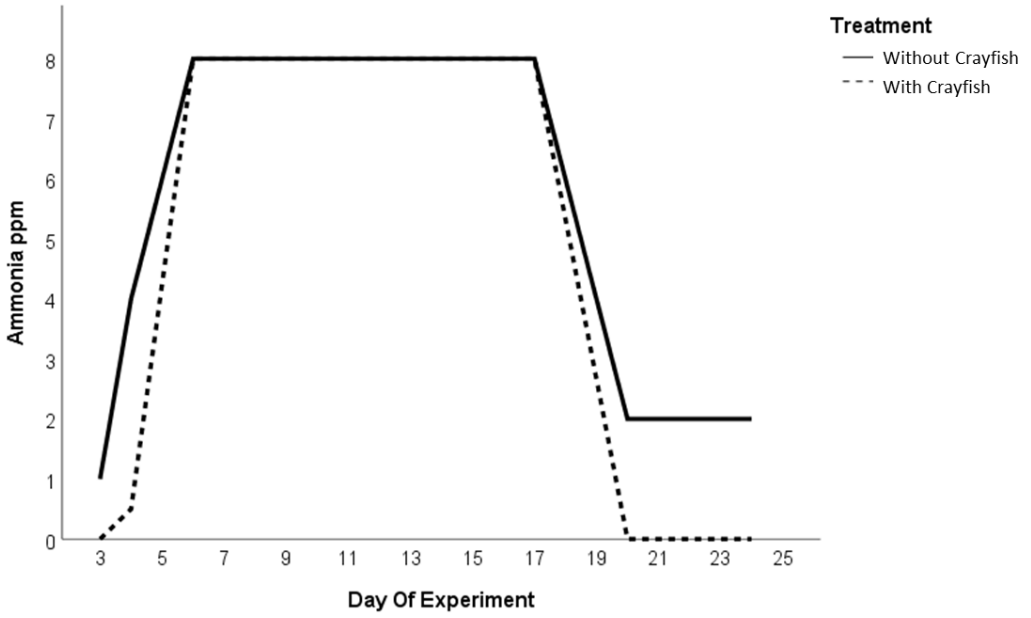

Figure 26. Ammonia levels per day of 24-day tank biofloc experiment for *Cherax quadricarinatus* nursery phase. Starter culture treatments were with redclaw crayfish and without redclaw crayfish.

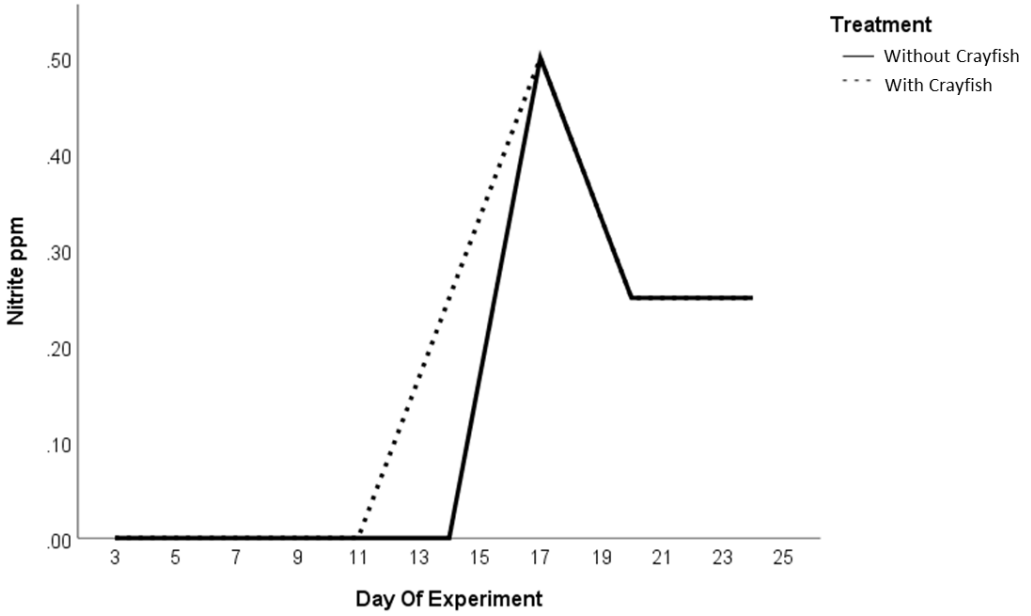

Figure 27. Nitrite levels per day of 24-day tank biofloc experiment for *Cherax quadricarinatus* nursery phase. Starter culture treatments were with redclaw crayfish and without redclaw crayfish.

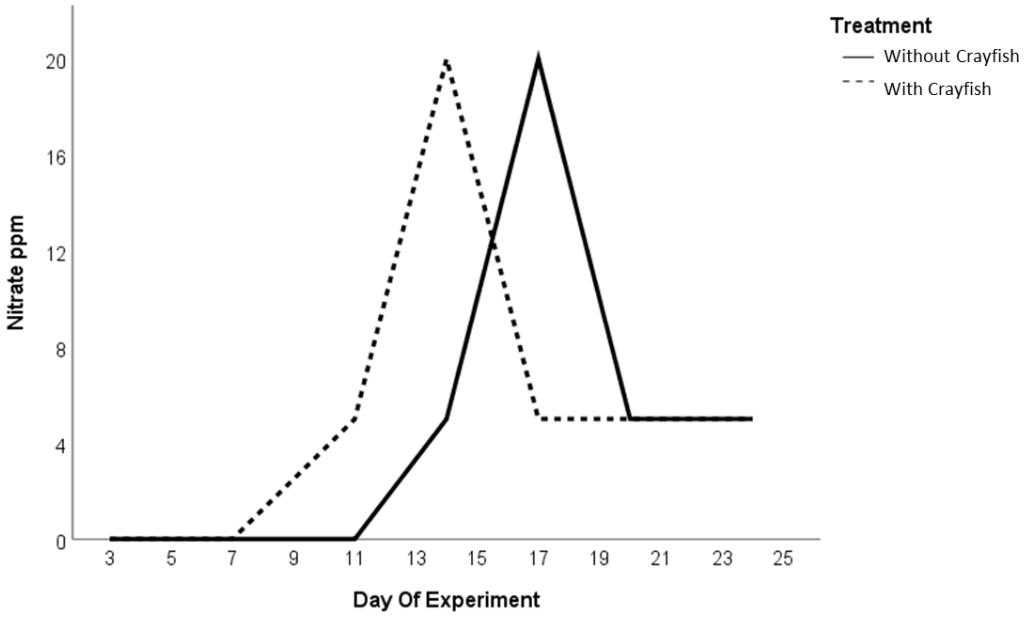

Figure 28. Nitrate levels per day of 24-day tank biofloc experiment for *Cherax quadricarinatus* nursery phase. Starter culture treatments were with redclaw crayfish and without redclaw crayfish.

Biofloc versus Clearwater. At the conclusion of the biofloc pilot study a large increase in mean wet weight was found for both treatments (Figure 30), however the total wet weight at the end showed that the Clearwater treatment was less than half of the starting wet weight, the BioFloc treatment had increased in total wet weight (Figure 31).

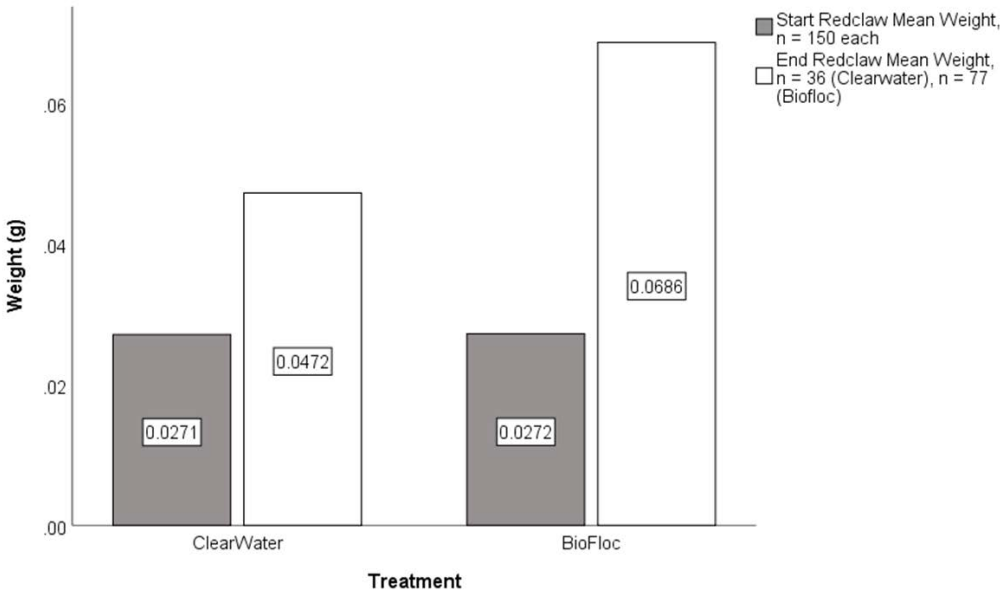

Figure 29. Start and end weight for 21-day tank biofloc experiment, Cherax quadricarinatus nursery phase, Clearwater versus Biofloc treatments.

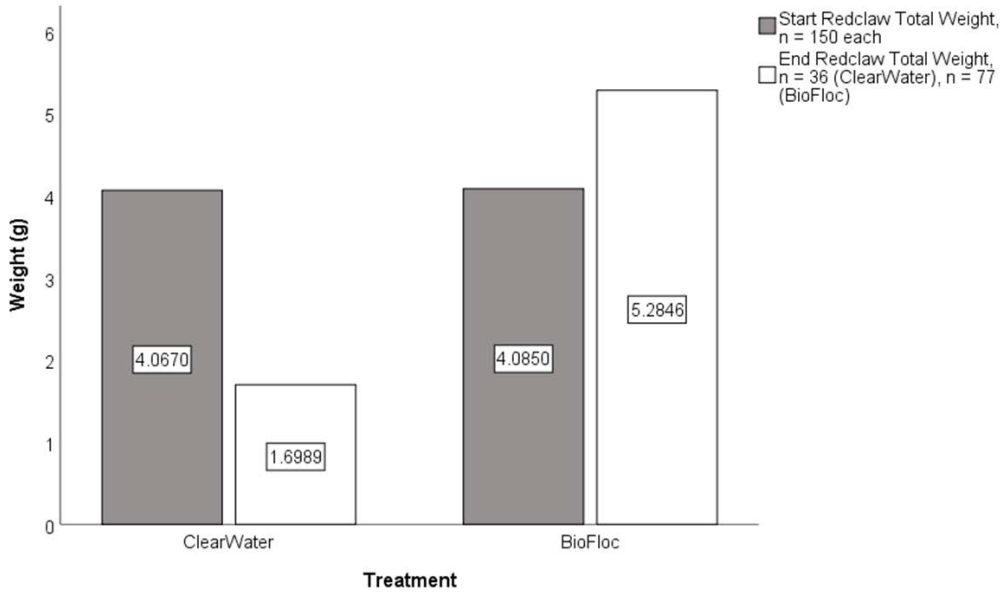

Figure 30 Total start and end weight for 21-day tank biofloc experiment, Cherax quadricarinatus nursery phase, Clearwater versus Biofloc treatments.

Both treatments started with 150 redclaw, at the conclusion of the experiment, the Clearwater treatment had 36 surviving, the Biofloc treatment had 77 survivors. At the end of the Biofloc experiment the survival percentage for the Biofloc treatment was twice that of the Clearwater treatment, the percentage weight change (mean) was higher

for the Biofloc than the Clearwater treatment, the percentage of original biomass was also higher for the Biofloc treatment (Figure 32).

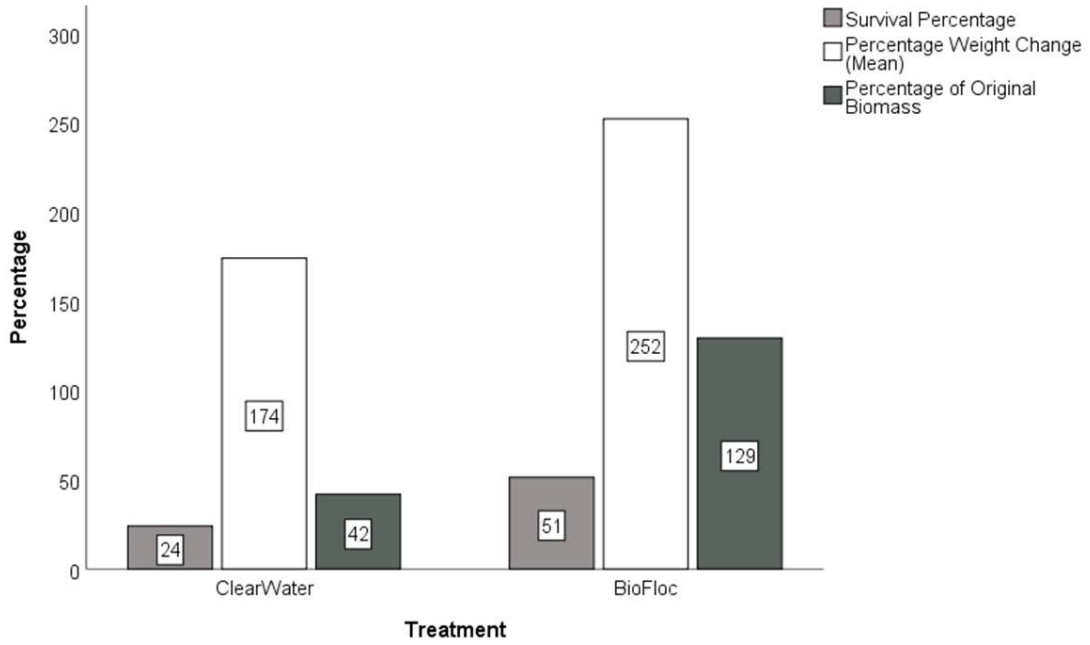

Figure 31. Survival percentage, percentage weight change and percentage of original biomass for 21-day tank biofloc experiment, *Cherax quadricarinatus* nursery phase, Clearwater versus Biofloc treatments.

6.3.4 Discussion

In the first section of the pilot study the treatment which included crayfish showed that the peak total ammonia nitrogen value occurred on day 14 as opposed to the treatment without crayfish which had a peak total ammonia nitrogen value at day 17. After these individual peaks there was evidence that nitrifying bacteria were present as the total ammonia nitrogen values immediately and dramatically decreased, and the ammonia levels decreased similarly at day 17 through to day 20. Ammonia remained high at 2 ppm for the remainder of the trial for the non-crayfish treatment. Nitrite levels peaked in both treatments on the same day, day 17, however the crayfish treatment had levels of Nitrite rising three days before. Nitrate levels peaked at a 3-day offset on day 14 for the crayfish treatment and day 17 for the non-crayfish treatment. The three-day

gap in peak values between the two treatments may be an artefact of the sampling design as readings were only taken every three days; the offset may well be less than this. More information and resolution of the data may be uncovered with more frequent sampling. Interestingly, the crayfish treatment which appeared to encourage the recruitment of nitrifying bacteria more quickly than the non-crayfish treatment, had far less production of floc at day 14 of the experiment, however this was the day that the total ammonia nitrogen peaked for this treatment and may be due to that.

This first part of the biofloc study gave evidence for two main conclusions. Firstly, the methods adapted from the literature allowed the production of biofloc, with or without crayfish within 14 days of commencing the trial. Secondly, the use of crayfish to help produce the biofloc appeared to shorten the time to produce the biofloc, and reduced ammonia, the most toxic portion of the ammonia cycle for redclaw, down to zero at day 20. It is apparent that the nitrogenous waste from the crayfish was critically important for the establishment and maintenance of nitrifying bacteria (Ulloa Walker 2020), forming a biofiltration system (Gutierrez-Wing and Malone 2006).

It would be of use to also measure floc production on day 17 when the non-crayfish treatment peaked in its total ammonia nitrogen value and also at the conclusion of the experiment for both treatments to compare. As this was a very preliminary trial to establish whether nitrifying bacteria and a floc could be established, a more robust trial taking into account daily sampling of water parameters and floc measurements, as well as replication, is warranted to provide higher data resolution.

In the second part of the study it is clear that there were benefits conveyed to the crayfish from the biofloc treatment in comparison with the clearwater treatment. The redclaw in the biofloc treatment had a larger end mean weight, a larger harvest total weight, a higher survival, a higher weight gain, and a higher biomass increase at

the end of the trial. It is apparent that the animals in the biofloc derived nutritional benefit from the biofloc in terms of survivorship and growth. Whether nutrition was assimilated via the digestive tract or whether the nutrition was absorbed via transport across the integument or gills or during ecdysis is unclear (Wheatly and Ayers 1995; Wheatly 1999; Shechter *et al*. 2008). A way to clarify this would be to repeat the same experiments with non-exogenous feeding lecithotrophic redclaw stages L1 and L2, to discover whether growth and survivorship is influenced by assimilation of 'dissolved organic matter (DOM)' (Salonen and Hammar 1986; Kankaala *et al*. 2010) nutrition assimilated from biofloc through means other than the digestive tract, through staining the DOM with a fluorescent marker and looking for evidence of it in the animals.

The pilot studies into tank biofloc production described here show promise as a way to enhance the nutrition of early instar redclaw, especially of those at J1 stage in a nursery phase. One of the advantages to conducting a nursery phase in grow-out ponds is the natural production which contributes to the growth and health of the redclaw (Jones 1995b). With further exploration of biofloc in tanks involving rigorous science and replication to perfect a methodology, biofloc may be the way to replicate the nutrition from grow-out ponds in tanks for a nursery phase and be added as a nursery phase protocol; something very common in shrimp aquaculture (El-Sayed 2021).

The ability to start a biofloc culture and the methodology for enabling this is valuable information produced by this study, as is the apparent contribution to growth of the biofloc. Survivorship was not as high as would be practical in a nursery phase conducted in tanks, a further avenue for investigation would be to trial a number of manufactured diets to test whether survivorship and growth could be improved, look at stocking density and habitat provision to combat cannibalism, and run the experiments at 22°C as Rigg *et al*. (2021a) showed significant mortality above 22°C. Nonetheless

biofloc shows strong potential for increased survivorship and growth, reduced cannibalism, reduced reliance on outside food sources and therefore cost, as well as benefits in terms of reduced water usage and disposal of nitrogenous waste to the environment.

6.4 Proximate Composition of Juvenile Redclaw; Protein and Lipid Content as Indices for Subsequent Growth and Survivorship

6.4.1 Introduction

Current redclaw aquaculture industry best practice involves stripping eggs from the female crayfish, followed by hatching and development to the third post-hatched instar (J1) conducted in a hatchery facility Jones and Valverde (2020). The subsequent output of animals weighing around 18 mg each are then shipped to the grow-out farmer and released into grow-out ponds. The grow-out ponds are generally around 1000 m^2 and are stocked with 10,000 J1's, (i.e.10 J1 per m^2) Jones and Valverde (2020). Apart from supplemental feeding, the redclaw are then left to grow to market size for around nine months. Predicting the outcome at harvest nine months ahead for animals that are released into a grow-out pond at *c.* 18 mg is difficult, especially given that their nutritional status and potential fitness for grow-out are unknown. A nursery phase, as proposed, is intended to add vigour to the J1 redclaw in order to ensure survivorship and good rates of growth, and add surety to the predicted yields and therefore the ability for a redclaw farmer to more ably budget for spending and income. Due to the relative lack of technical sophistication of the redclaw aquaculture industry at present, it may be advantageous to look to aquaculture practices from other industries to adapt and use for redclaw. Some sort of measure of condition which may illustrate the potential for growth and survival of the crayfish, or which may quantify the effect of a treatment

factor such as different diets, would be an excellent tool for the aquaculture industry of redclaw.

It has been demonstrated that marine rock lobster pueruli (the final larval stage) have a store of lipids that is used as an energy source to fuel their active swimming, as they seek out suitable habitat on which to settle (Jeffs *et al.* 1999). The quantum of lipid in the body can therefore be used as a measure of condition (Jeffs *et al*. 1999). This project aimed to measure the utility of lipid and protein composition of redclaw juvenile metrics as a proxy for physiological condition, following a previously established method (Jeffs *et al.* 1999). Further, we aimed to determine the relative efficacy of body protein and/or lipid proportion as a predictor for growth and survival and if this is reflected in the effect of various diet treatments on growth and survival.

6.4.2 Methods

A diet trial was conducted over six weeks at James Cook University, Cairns campus. Instar J1 redclaw were sourced from AquaVerde Redclaw Farm, and placed in recirculating aquaculture systems consisting of four individual systems comprising a separate sump and five experimental tanks. Animals were placed in tanks at the rate of 47 to 54 per tank and weighed for wet weight *en masse* to establish a mean wet weight for each experimental tank. The treatment for the experiment was different diets, comprising; a manufactured diet (CSIRO formulation), frozen defrosted bloodworms, frozen defrosted *Artemia* sp. and a commercial larval shrimp diet (Frippak), fed daily to satiation. Details of these diets and apparatus are provided in Chapter 2, "Evaluation of Four Practical Diets on the Growth and Survival of Juvenile Redclaw, *Cherax quadricarinatus* (von Martens, 1868)". In this trial however, the redclaw were held in tubs without individual baskets or habitat.

At weeks four and six a random subsample of the crayfish was taken for analysis for protein and lipid composition (for each of the four diet treatments; $n = 10$ at four weeks, $n = 7$ at six weeks). The week four sampling was opportunistic as other samples were sent at the time. It was also thought that it would provide interesting data to see if these parameters changed over the course of two further weeks as the animals developed and moved through instars.

All remaining animals were then weighed for wet weight at the six-week conclusion of the experiment. Samples were lyophilised for a minimum of three days and sent to The National Institute of Water and Atmospheric Research (NIWA) in New Zealand for determination of lipid and protein weight.

Upon receipt at NIWA, each frozen crayfish was measured with calipers to ascertain occipital carapace length, the dry weight was recorded, and then all appendages were removed to validate comparisons between those with missing limbs and those without (Jeffs *et al.* 1999) (for each of the four diet treatments; $n = 10$ at four weeks, $n = 7$ at six weeks). The individual crayfish bodies were ground to a fine powder in a liquid nitrogen cooled pulveriser and divided into weighed aliquots for separate assays for total protein and total lipid, and again divided into duplicates for each crayfish (Jeffs *et al.* 1999). Duplicates for each crayfish were run and means generated. The total protein figure was quantified using the Bicinchoninic Acid Reagant (Sigma, USA), spike and recovery techniques verified the result (Jeffs *et al*. 1999).

6.4.3 Results

The mean wet weight for the treatments at the start of the experiment were not significantly different from each other $F_{3, 16} = 1.305$, $p = 0.307$, nor were there any significant differences based on wet weights at the start of the experiment for system effect $F_{3, 16} = 1.396$, $p = 0.280$, or tank effect $F_{4, 15} = 1.424$, $p = 0.274$.

Week four data shows the Frippak diet treatment produced animals with significantly longer ocular carapace length $F_{3, 36} = 3.837$, $p = 0.018$, $n = 10$, significantly higher lipid weight $F_{3, 36} = 8.210$, $p < 0.001$, $n = 10$ and significantly higher dry (lyophilised) weight $F_{3, 36} = 11.513$, $p < 0.001$, $n = 10$ (Figure 33) than the other three diet treatments.

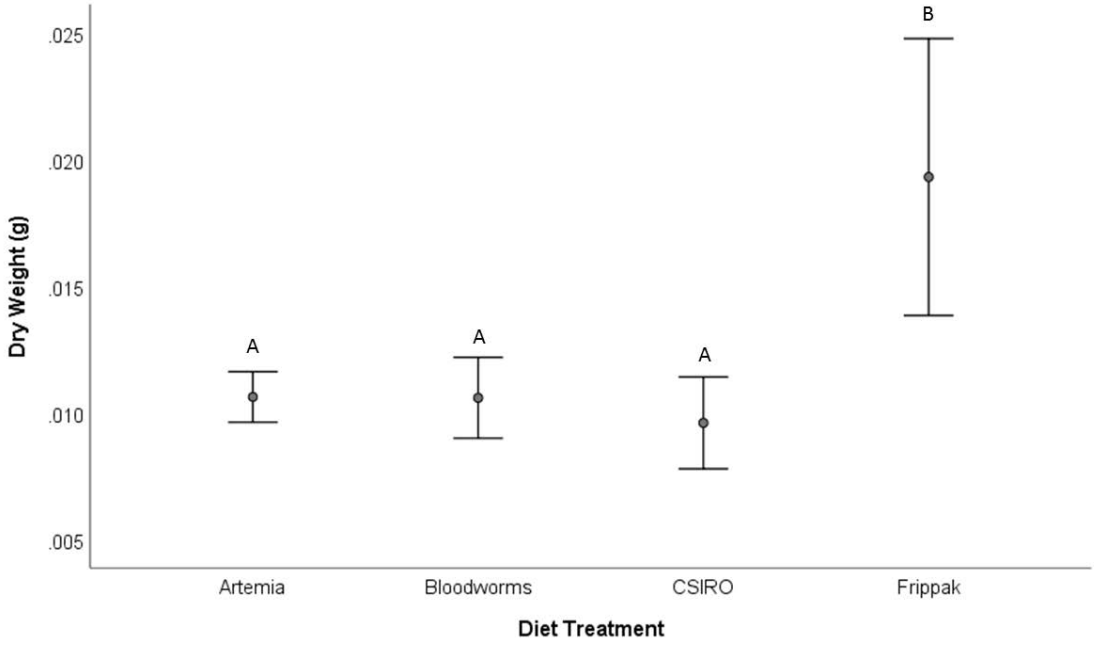

Figure 32. Dry (lyophilised) weight of *Cherax quadricarinatus* fed one of four diet treatments for four weeks.
Treatments with the same letter are not significantly different. Error bars represent 95% Confidence Interval, $n = 10$ for each treatment.

Week four data for the proximate composition experiment, showed bloodworms diet crayfish were significantly higher than *Artemia* sp. diet crayfish in percentage of lipids per dry (lyophilised) weight ($p = 0.011$), but not significantly different to CSIRO diet ($p = 0.053$) and Frippak diet crayfish ($p = 0.472$). *Artemia* sp. crayfish are significantly lower than bloodworms diet ($p = 0.011$) but not significantly different to

CSIRO diet ($p = 0.503$) and Frippak diet crayfish ($p = 0.059$) ($F_{3, 36} = 2.931$, $p = 0.047$, $n = 10$) (Figure 34).

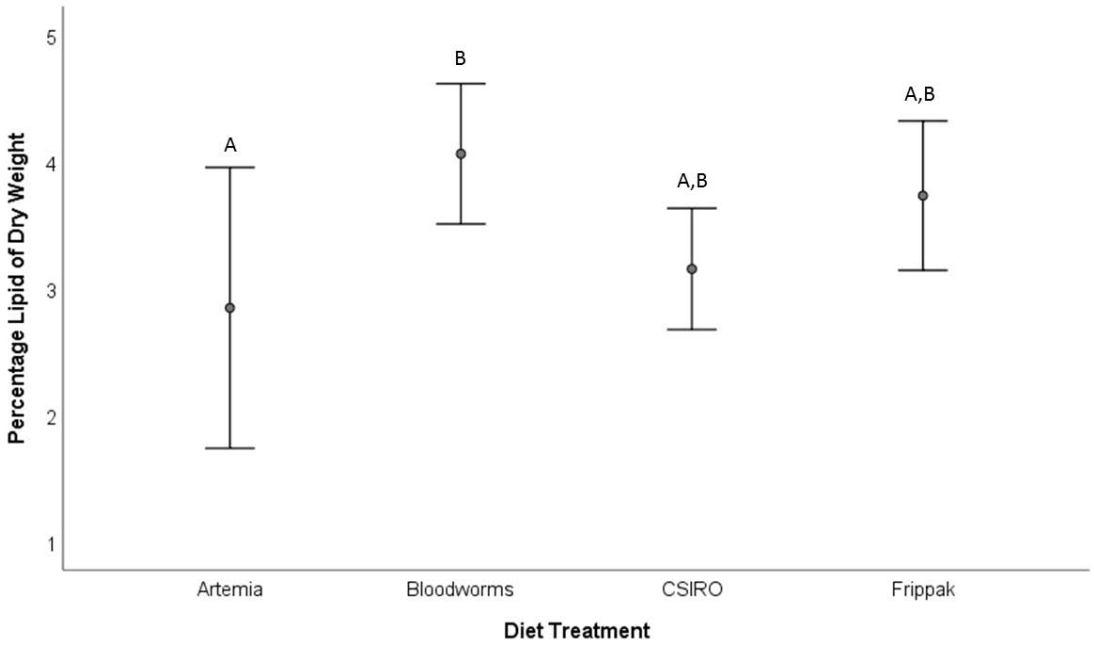

Figure 33. Percentage lipid of dry (lyophilised) weight of *Cherax quadricarinatus* fed one of four diet treatments for four weeks.
Treatments with the same letter are not significantly different. Error bars represent 95% Confidence Interval, $n = 10$ for each treatment.

At week four Frippak was significantly higher in weight of protein than the other three diets, which did not differ significantly from each other $F_{3, 36} = 7.725$, $p < 0.001$, $n = 10$ for each treatment (Figure 35).

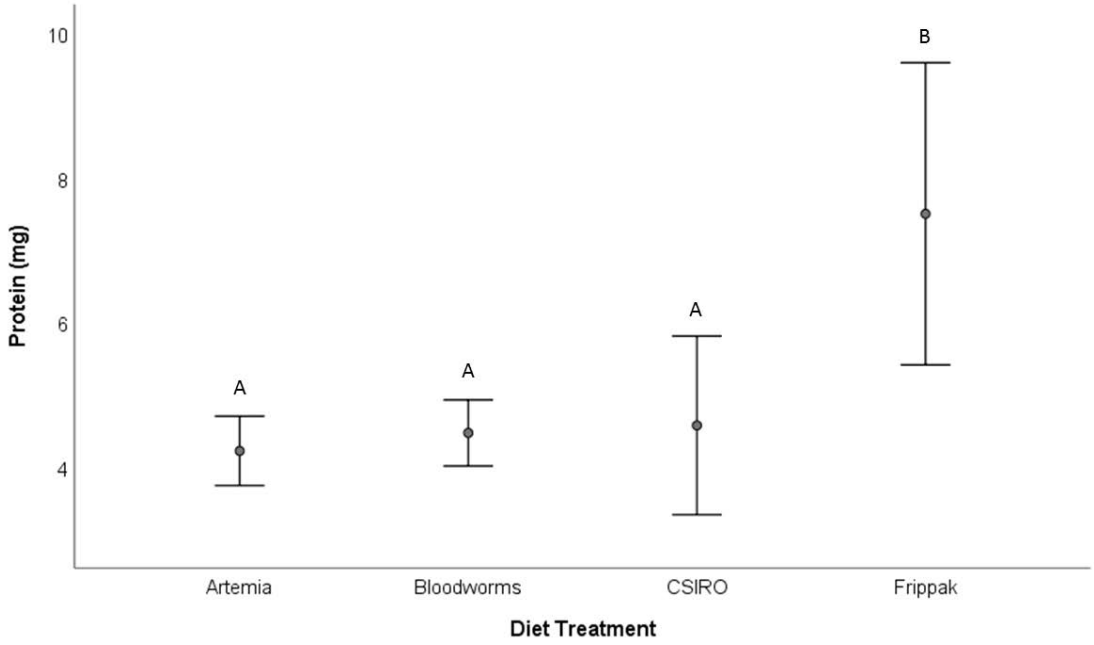

Figure 34. Protein weight for *Cherax quadricarinatus* fed one of four diet treatments for four weeks.
Treatments with the same letter are not significantly different. Error bars represent 95% Confidence Interval, $n = 10$ for each treatment.

After six weeks the Frippak diet treatment redclaw were significantly longer in ocular carapace length $F_{3, 24} = 3.352$, $p = 0.036$, $n = 7$ for each treatment, significantly higher in wet weight $F_{3, 24} = 5.791$, $p = 0.004$, $n = 7$ for each treatment, significantly higher in dry (lyophilised) weight $F_{3, 24} = 7.946$, $p = 0.001$, $n = 7$ for each treatment (Figure 36), significantly higher in lipid weight $F_{3, 24} = 5.939$, $p = 0.004$, $n = 7$ for each treatment (Figure 37), and significantly higher in protein weight than the other three diet treatments $F_{3, 24} = 5.226$, $p = 0.006$, $n = 7$ for each treatment (Figure 38).

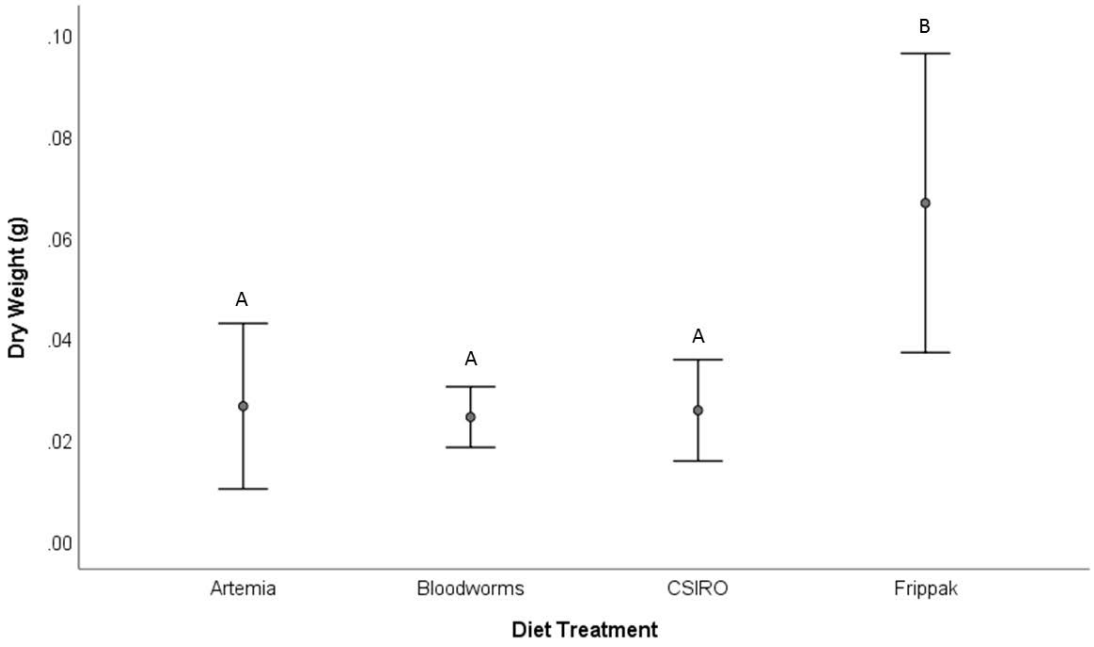

Figure 35. Dry (lyophilised) weight of *Cherax quadricarinatus* fed one of four diet treatments for six weeks.
Treatments with the same letter are not significantly different. Error bars represent 95% Confidence Interval, $n = 7$ for each treatment.

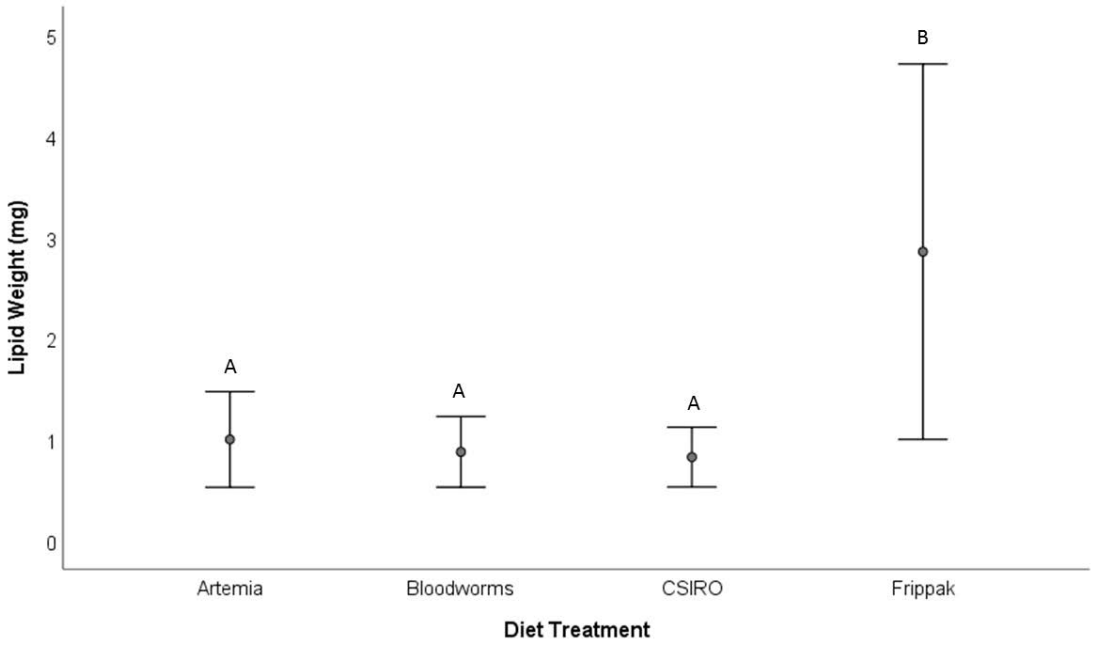

Figure 36. Lipid weight of *Cherax quadricarinatus* fed one of four diet treatments for six weeks.
Treatments with the same letter are not significantly different. Error bars represent 95% Confidence Interval, $n = 7$ for each treatment.

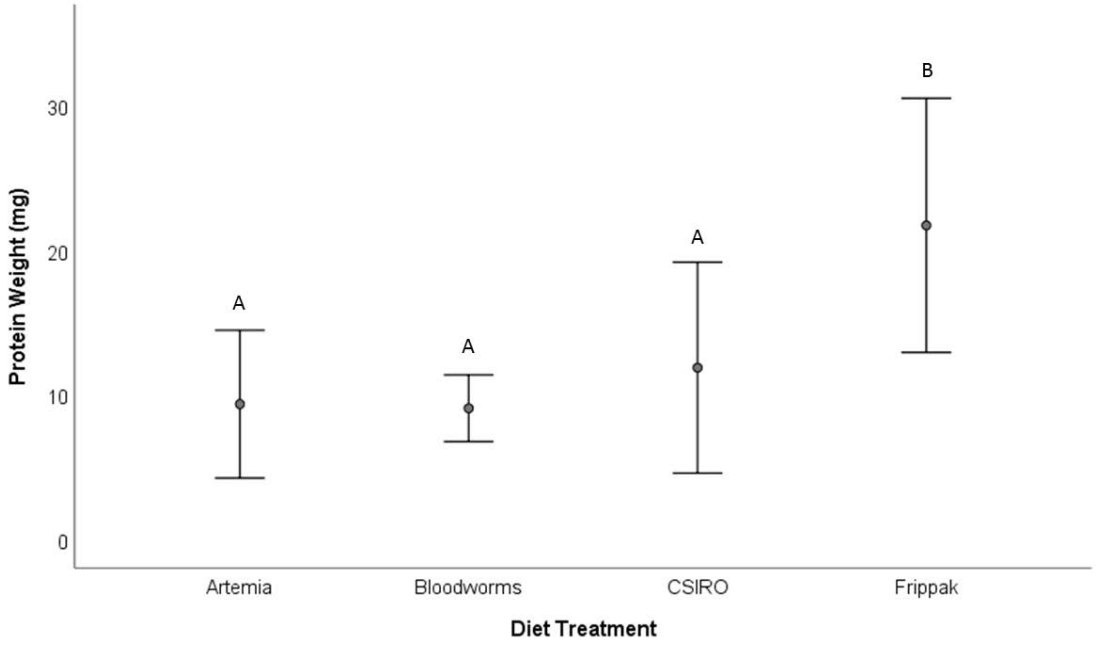

Figure 37. Protein weight of *Cherax quadricarinatus* fed one of four diet treatments for six weeks.
Treatments with the same letter are not significantly different. Error bars represent 95% Confidence Interval, $n = 7$ for each treatment.

Redclaw fed one of four diet treatments for six weeks indicated that the CSIRO diet was significantly higher in percentage of protein per dry weight than all but bloodworms $F_{3, 24} = 3.563$, $p = 0.029$, $n = 7$ for each treatment (Figure 39).

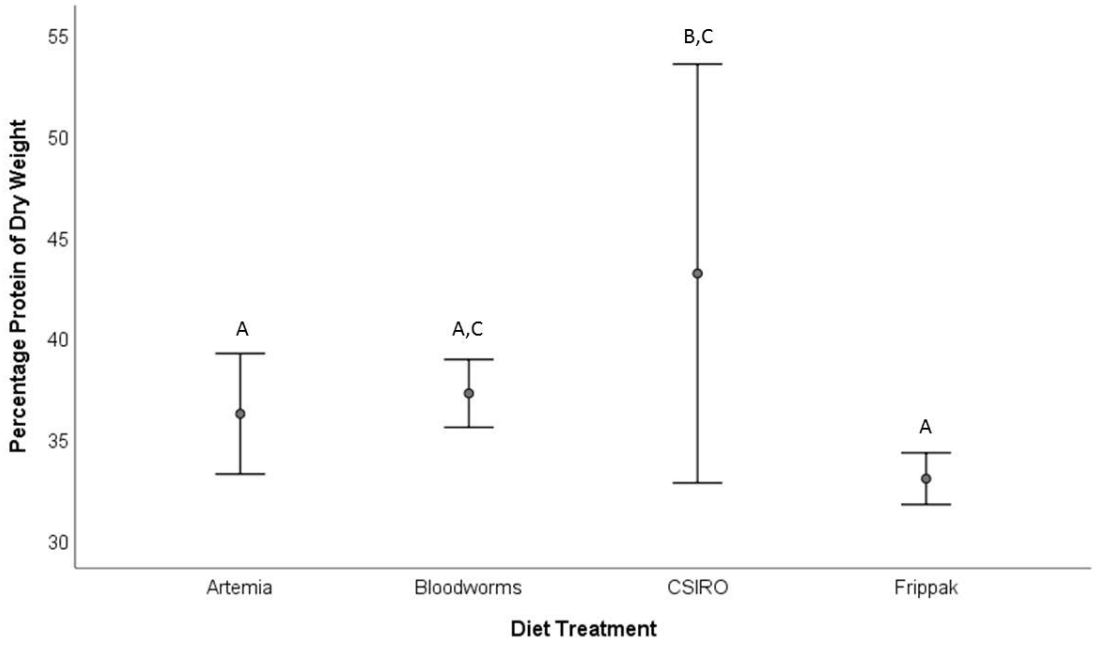

Figure 38. Percentage protein of dry (lyophilised) weight *Cherax quadricarinatus* fed one of four diet treatments for six weeks.
Treatments with the same letter are not significantly different. Error bars represent 95% Confidence Interval, $n = 7$ for each treatment.

The percentage change across the period from four to six weeks was calculated for ocular carapace length, dry weight, wet weight, lipid weight, percentage of lipid of dry weight, protein weight and percentage protein of dry weight (Figure 40). In all of these parameters except percentage lipid of dry weight and percentage protein of dry weight, Frippak showed the largest increase compared to the other three diet treatments, week four, $n = 10$ for each treatment, week six, $n = 7$ for each treatment (Figure 40).

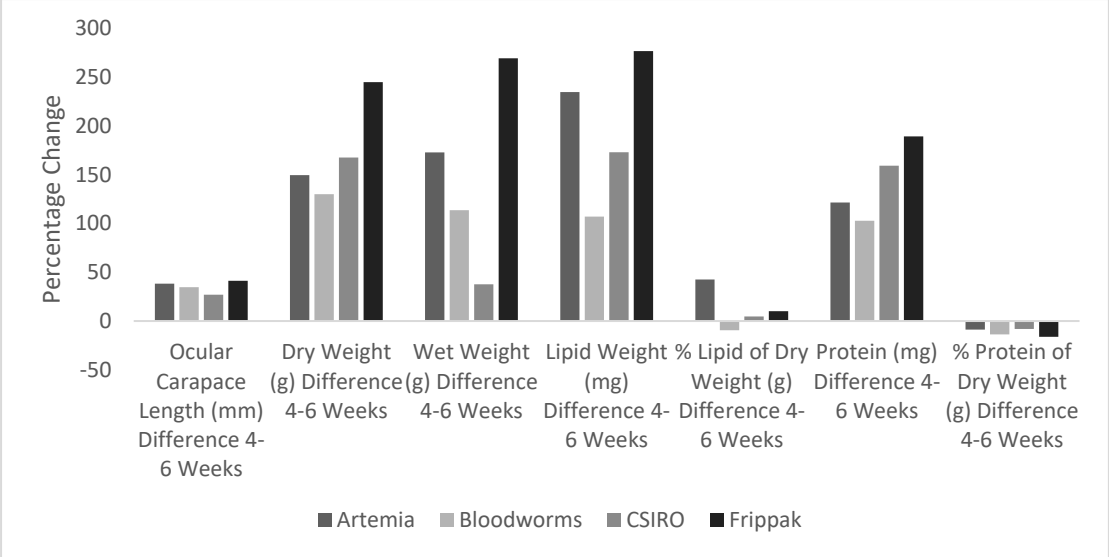

Figure 39. All measured parameters of *Cherax quadricarinatus* fed one of four diet treatments, change between weeks four and six data.

Total biomass for all the surviving animals at the conclusion of the trial at six weeks showed that CSIRO diet fed redclaw had the highest total biomass, followed by the Frippak diet fed redclaw (Figure 41). Redclaw fed the *Artemia* sp. diet had 24 survivors (9.4% survival), bloodworms fed redclaw also had 24 survivors (9.4% survival), CSIRO fed redclaw had 42 survivors (16.7% survival), and Frippak fed redclaw had 14 survivors (5.5% survival) at the conclusion of the trial after six weeks (Figure 41). The mean wet mass of all the surviving animals after six weeks showed that Frippak diet fed redclaw were significantly heavier than redclaw fed the other three diets which did not differ from each other, $F_{3, 100} = 27.448$, $p < 0.001$, *Artemia* sp. n = 24, bloodworms n = 24, CSIRO n =42, Frippak n =14 (Figure 42).

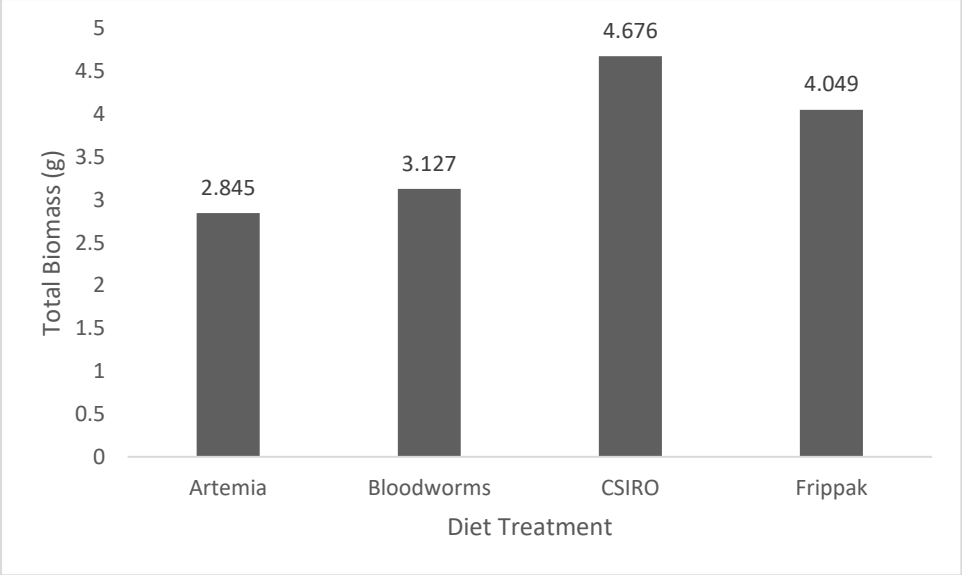

Figure 40. Total biomass of all surviving *Cherax quadricarinatus* fed one of four diet treatments for six weeks, *Artemia n* = 24, Bloodworms *n* = 24, CSIRO *n* = 42, Frippak *n* = 14.

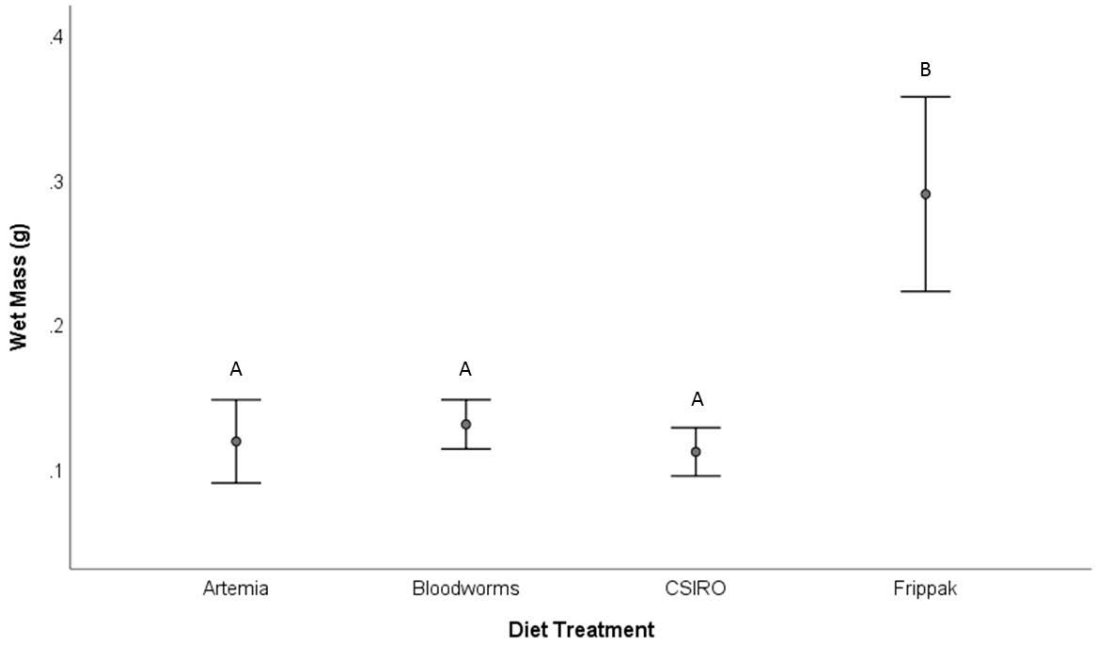

Figure 41.Wet mass of all surviving *Cherax quadricarinatus* fed one of four diet treatments for six weeks.
Treatments with the same letter are not significantly different. Error bars represent 95% Confidence Interval, *Artemia n* = 24, Bloodworms *n* = 24, CSIRO *n* = 42, Frippak *n* = 14.

6.4.4 Discussion

This study aimed to discover whether any of the parameters which were measured could be used as a proxy for condition. Ocular carapace length, dry weight and wet weight were the parameters measured to indicate growth in this study, and in all those categories, except at week four for wet weight (no significant difference between diet treatments), the Frippak diet treatment produced significantly larger redclaw. Matching these data with the data collected to potentially indicate growth, redclaw fed the Frippak diet treatment also had the significantly highest lipid weight and protein weight at weeks four and six and amongst the surviving redclaw at the end. This result is intuitive, as the Frippak diet redclaw were larger in all the parameters measured, therefore it would be expected that the absolute weight of lipids and proteins in these animals would be greater.

Examining the proportion of lipids as percentage of dry (lyophilised) weight of redclaw in the study shows that at week four the redclaw fed the bloodworms were significantly higher than redclaw fed the *Artemia* sp. but not significantly different to the other two diet treatments. At week six there was no statistical difference between treatments, and the percentage change from four to six weeks in redclaw fed the *Artemia* sp. had the highest percentage change in lipid percentage of dry weight. Comparing the growth data in terms of wet weight, dry weight and ocular carapace length, redclaw fed the Frippak diet were significantly highest across weeks four (no statistical difference in wet weight), week six and for wet weight in the surviving animals. As bloodworms or *Artemia* sp. fed redclaw had the highest amount of percentage of lipids of dry weight, it would appear that percentage lipids of dry (lyophilised) weight is not a good index for condition of growth. As far as survivorship compares to percentage lipids of dry weight, the CSIRO treatment produced the highest survivorship of any of the diet

treatments, albeit still extremely low, followed by the *Artemia* sp. and bloodworms treatments. As redclaw fed the bloodworms or *Artemia* sp. had the highest amount of percentage of lipids of dry weight, in this instance lipids percentage dry weight would not appear to be a good index for survival either.

The proportion of protein as percentage of dry (lyophilised) weight of redclaw in the study shows that at week four there was no significant difference between diet treatments. At week six redclaw fed the CSIRO treatment were significantly higher than all other treatments except bloodworms, and in the animals remaining at the end of the study the least negative change in percentage lipid of dry weight were redclaw fed the CSIRO diet. The Frippak diet redclaw were significantly highest in terms of wet weight, dry weight and ocular carapace length across weeks four (no statistical difference in wet weight), week six, and in the surviving animals. Therefore it would appear that percentage protein of dry weight is also not a good index for condition of growth. The CSIRO diet treatment produced the best survivorship for any of the diet treatments, albeit still extremely low, so perhaps the percentage proportion of protein of dry weight may be a useful index for survival. Further tests should be done to establish this with an experiment with far higher survival rates.

One interesting result from the percentage change in protein of dry weight between weeks four and six, was that the percentage in all cases was negative. This means that in the two weeks after week four they decreased in protein content per gram dry weight. If the body composition is reflective of the nutritional requirements for the crayfish this may signal an early ontogenetic diet change towards lower protein in the diet. It would be of value to redclaw aquaculture farmers to ascertain if this is the case, as a lower protein requirement translates to a lower price for feed and therefore lower overall cost. Perhaps one way to achieve this is to examine α-amylase cellulose

laminarinase activity in the redclaw alimentary tract (Figueiredo *et al*. 2001; Campaña-Torres *et al*. 2008) and the presence of *p*-nitrophenyl glycosidases in the gastric fluids (Figueiredo *et al*. 2001; Campaña-Torres *et al*. 2008) as it has been demonstrated that redclaw can assimilate cellulose (Xue *et al*. 1999; Pavasovic *et al*. 2006; Campaña-Torres *et al*. 2008) and this may signal the shift to a more plant-based diet.

The effect of cannibalism confounds the result here, in that the measured parameters for growth i.e. ocular carapace length, wet weight and dry weight for the most part reflected the Frippak diet treatment redclaw which experienced the highest cannibalism. It is not known whether it was the diet which enhanced the growth in these redclaw, or whether the nutrition they gained from cannibalism was the driver of the larger measured parameters and ultimately the larger animals. It is difficult to extract meaningful data on growth and survivorship in this case as there was 94.5% mortality, and it is unknown whether the cannibalism was the cause of the mortalities or whether the animals were cannibalised after death. In either case it is very difficult to attribute growth to the Frippak diet alone. This is also the case but in a slightly lesser fashion for the other diet treatment redclaw.

The percentage protein of dry weight may hold promise for an index of condition for survivorship, in that the percentage protein dry weight at weeks six and in the surviving animals tends to indicate that the CSIRO diet treatment produced the highest survivorship by promoting higher percentage protein. There is however thin evidence for this, but a meaningful study in which cannibalism can be excluded as per the methods in Rigg *et al* 2021 may uncover clearer evidence of the efficacy of using these measurements as indices of condition.

As seen in this study, the one potentially confounding effect in many studies conducted with redclaw is the effect of cannibalism. It is not always possible to separate

the nutrition that the redclaw receive from the feed given rather than the nutrition they gain from consuming conspecifics. In a farming context it would be impractical to hold redclaw individually, therefore one of the most important parameters to explore for a nursery phase conducted in tanks is the density they can be stocked at whereby cannibalism is minimised. Coupled with density parameters a provision of habitat shows promise to help mitigate cannibalism too, the design and quantity of which should be explored.

6.5 Effect of Stocking Density and Shelter on Culture of Juvenile Redclaw *Cherax quadricarinatus*

6.5.1 Introduction

The current hatchery technology that exists for redclaw allows for an aquaculture farmer to order seedstock from a hatchery for stocking of grow-out ponds. The animals supplied are of very similar ages and instar, J1 at around 18mg and numbering 10,000 to stock a 1,000 m^2 grow-out pond. If they were to disperse with uniform space between them they would occupy the pond at ten J1 per m^2 in the two-dimensional benthic space. The pond size is suited to the larger animals as they grow and designed to accommodate potentially 10,000 adult redclaw at harvest. As a consequence, this initial stocking density of J1 instar redclaw is very low and the potential for aggressive interactions and cannibalism of conspecifics are presumably reduced due to the low density. The initiation of a nursery phase in tanks requires maximum density to offset costs involved with intensive husbandry, which means that aggressive interactions and cannibalism need to be mitigated for, to increase survivorship. As observed and noted in Sections 6.3 (Application of Biofloc to redclaw nursery phase) and 6.4 (Proximate composition of juvenile redclaw protein and lipid content as indices for growth and survivorship) of this chapter, cannibalism over a number of weeks can reduce survivorship dramatically. One method to mitigate cannibalism and aggressive interactions due to high stocking density is to provide a form of habitat or structure for individuals to take shelter in. In the grow-out ponds, habitat is provided in the form of stacks of mesh tubes for larger animals and onion bag mesh or similar for smaller animals (Jones 1990). This study attempted to uncover the highest stocking density possible in tanks whilst mitigating cannibalism, trialing two

types and two sizes of suitably-sized scaled-down habitat for J1 redclaw in a nursery phase.

Redclaw is essentially a benthic animal which means that in effect it is using two-dimensional space (Barki *et al*. 2006). However, by creating greater complexity in their environment through the addition of more substrate, a third dimension can be utilised and greater densities supported. Maximising density is critical to the redclaw farmer to gain greatest economic benefit from the pond infrastructure. Equally, too high a stocking rate can result in density-dependent growth inhibition (Karplus and Barki 2004), where the size of animals is constrained by lack of space, cannibalism may be increased due to the close proximity of neighbours whilst performing ecdysis (Barki *et al*. 1997), and aggressive interactions can result in damaged animals and lower value.

Stocking density can have effects throughout grow-out (Pinto and Rouse 1996; Jones and Ruscoe 2000; Naranjo-Paramo *et al.* 2004) including yield at harvest (Allan and Maguire 1992; Geddes *et al*. 1993; Daniels *et al*. 1995; Morrissy *et al*. 1995; Tidwell *et al*. 1999) and weight at harvest whereby the relationship is often inversely related to original stocking density (Pinto and Rouse 1996; Jones and Ruscoe 2000; Barki and Karplus 2004; Karplus and Barki 2004; Naranjo-Paramo *et al*. 2004; Rodgers *et al.* 2006). Habitat or shelter may contribute to increased growth rates (Karplus *et al*. 1995) and assist in reducing the negative effects of high stocking density (Jones and Ruscoe 2000). To maximise stocking rate redclaw farmers provide grow-out ponds with habitat to provide a means for safely separating the animals from each other, shielding them from aggressive interactions and cannibalism (Jones 1990, 1995b; Barki *et al*. 1997).

A number of different materials were tried and tested for habitat in the early development of the redclaw aquaculture industry, including used car tyres, sections of

PVC pipe, cement sheeting and onion bag mesh (Jones 1995c; Jones and Ruscoe 2001; Viau and Rodríguez 2009). The commonly provided habitat presently consists of plastic mesh tubes bundled together and stacked offset on top of each other. Onion bag mesh was used early on in redclaw farming as it was found that when breeding redclaw in ponds, juveniles would gather on and in the onion bag mesh using it as safe habitat, keeping them separate from conspecifics. Following on from some pilot studies, (D. Rigg, unpublished) it was decided that a replication in miniature of the onion bag mesh and plastic mesh tubes may be suitable for habitat / shelter for J1 redclaw in a nursery phase. In other experiments (Rigg *et al*. 2021a, b) redclaw were by necessity held individually so as to prevent cannibalism and track individual development and survivorship. As the proposed nursery phase for redclaw in these trials follow on from the hatchery in an intensive, indoor situation, the maximisation of space including use of the three-dimensional space is of paramount importance; as is the prevention of cannibalism. Holding redclaw individually in a nursery phase would be impractical and costly, therefore a habitat which protects against cannibalism and allows for high levels of growth and survivorship is extremely important.

The aims of this study were to identify the stocking density and habitat requirement for redclaw J1 within a nursery phase to maximise the stocking density and use of the three-dimensional space of the experimental tanks while negating cannibalism.

6.5.2 Methods

AquaVerde redclaw farm in North Queensland (17°24'20.0" S, 145°31'32.8" E) supplied redclaw J1 from four broodstock females which were mixed to randomly assign to treatments and then acclimated at 22°C for two days in the laboratory at James Cook University prior to each of the two experiments. The redclaw were held at this temperature as prior experiments (Rigg *et al*. 2021a) established this as the most suitable temperature for a combination of growth and survivorship in laboratory conditions. Experimental animals were individually placed on kitchen paper for three seconds to remove surface water then weighed at the start and at the end of each experiment.

Initially feeding was at a rate which was in excess and blocked the outlet screens in the tanks with the potential to reduce water quality. Feed was reduced to a lower rate, which still allowed animals to be fed *ad libitum*, to satiation. Feeding protocol was; five cubes of defrosted frozen *Artemia* sp. (Aqua One©) (total wet weight 17.9 g) were mixed with aged, aerated tap water to make a solution of 50 ml, loaded into a 3 ml syringe and fed out at the rate of 1ml for treatments with 18 redclaw per tank, 2 ml for treatments with 36 animals, and 3 ml for treatments with 72 animals. Redclaw were fed *ad libitum* daily in the afternoon in both experiments. For details of experimental apparatus please refer to the Appendix, Figures 44 – 49.

Table 6. Habitat and density experiment treatments.

Microhabitat Type	Stocking Density redclaw / m^2 (includes bottom surface area of tank plus area of habitat)	Redclaw per Tank	Replicates (number of tanks)
Large Bowtie	11	18	3
Small Bowtie	13	18	3
Small Bowtie	26	36	3
Small Bowtie	52	72	3
Long Tube	13	18	2
Short Tube	14	18	2
Long Tube	21	36	3
Short Tube	26	36	3
Long Tube	32	72	3
Short Tube	43	72	3

The tube microhabitat arrangements were constructed of 12 drinking straws held inside 2.54 cm (1") diameter plastic pipe. The tube microhabitats were of two sizes, 2cm long (Short Tube) and 4cm long (Long Tube), for photographic reference please refer Appendix, Plate 6, tube microhabitat.

For the bowtie microhabitat experiment, dressmaker's tulle was measured and proportioned to be comparable to the microhabitat treatment provisions in the tube microhabitat experiment. The material was arranged and clumped so that it provided 3-

dimensional space, with a pair of fishing sinkers attached to provide negative buoyancy. The two sizes of bowtie microhabitat were 8cm^2 (Small Bowtie) and 16cm^2 (Large Bowtie), for photographic reference please refer Appendix, Plate 7, bowtie microhabitat.

In the tube microhabitat experiment, a water exchange of approximately 25% was performed daily to maintain water quality; 20 L systems 1 – 4 (3 tank systems), and 15 L for systems 5 and 6 (2 tank systems). In the bowtie microhabitat treatments this was changed to a 20 L water exchange for all systems, as they were all 3 tank systems. Water temperature was maintained at 22°C +/- 0.5°C via heater / chiller units in the sumps, air stones in the sumps provided aeration of the water. Ammonia and pH readings were taken daily throughout the experiment using an API Freshwater Master Test Kit, Nitrite was measured weekly. Dissolved oxygen readings were taken weekly via YSI Professional Plus water quality meter (Yellow Springs Instrument Company Ohio USA).

At the completion of each experiment Statistical analysis was conducted in IBM SPSS Statistics 24. Data were analysed via One Way ANOVA for density treatment effect on total percentage weight gain, significant differences were further explored through least significant difference (LSD) analysis. Data for density treatment effect on survival percentage were analysed via a One Way ANOVA, significant differences were further explored through least significant difference (LSD) analysis. Data were analysed via One Way ANOVA for microhabitat treatment effect on total percentage weight gain, significant differences were further explored through least significant difference (LSD) analysis. Data for microhabitat treatment effect on survival percentage were analysed via a One Way ANOVA, significant differences were further explored through least significant difference (LSD) analysis.

6.5.3 Results

For the duration of the experiment ammonia was maintained at ≤ 0.5ppm, pH was maintained at 7.2 – 7.6, nitrite remained at 0 and dissolved oxygen was 90% - 100%. Stocking density did not significantly affect weight gain or significantly affect percentage survival. Microhabitat treatment significantly affected percentage survival, whereby the two variants of the tube habitat promoted increased survival $F_{3, 27} = 6.668$, $p = 0.002$ (Figure 42). Microhabitat treatment significantly affected percentage weight gain, whereby the bowtie habitats promoted increased weight gain compared to the short tube habitat, $F_{3, 27} = 4.251$, $p = 0.015$ (Figure 43).

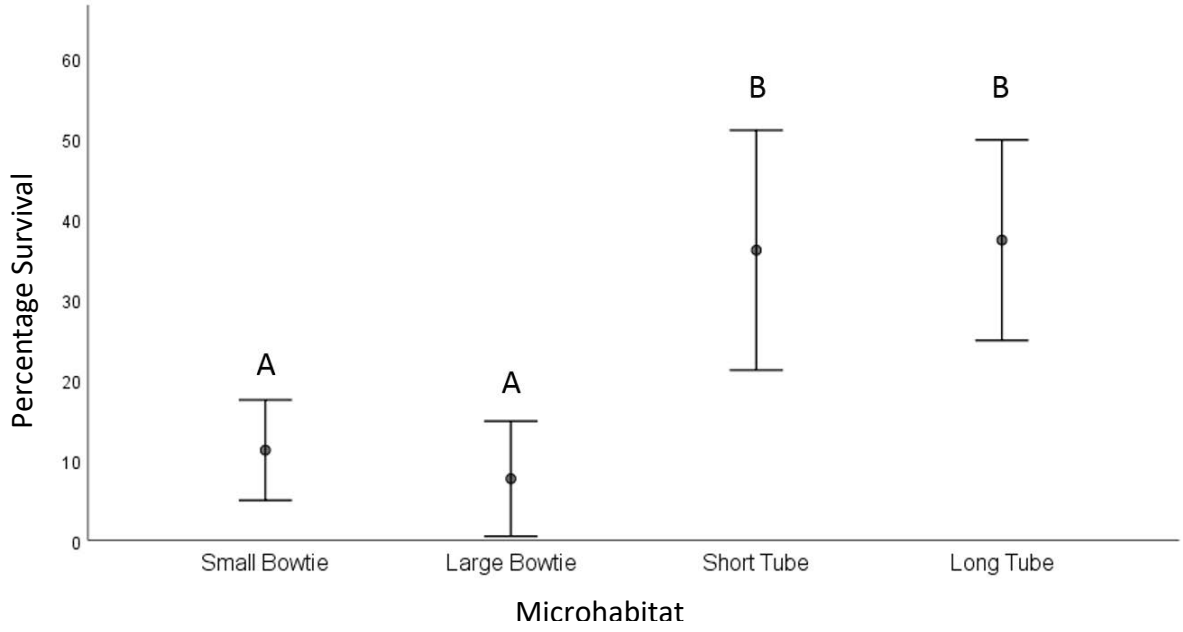

Figure 42. The percentage survival of J1 *Cherax quadricarinatus* exposed to one of four microhabitat treatments.

Error bars indicate the 95% Confidence Interval, corresponding letters indicate non-significant differences.

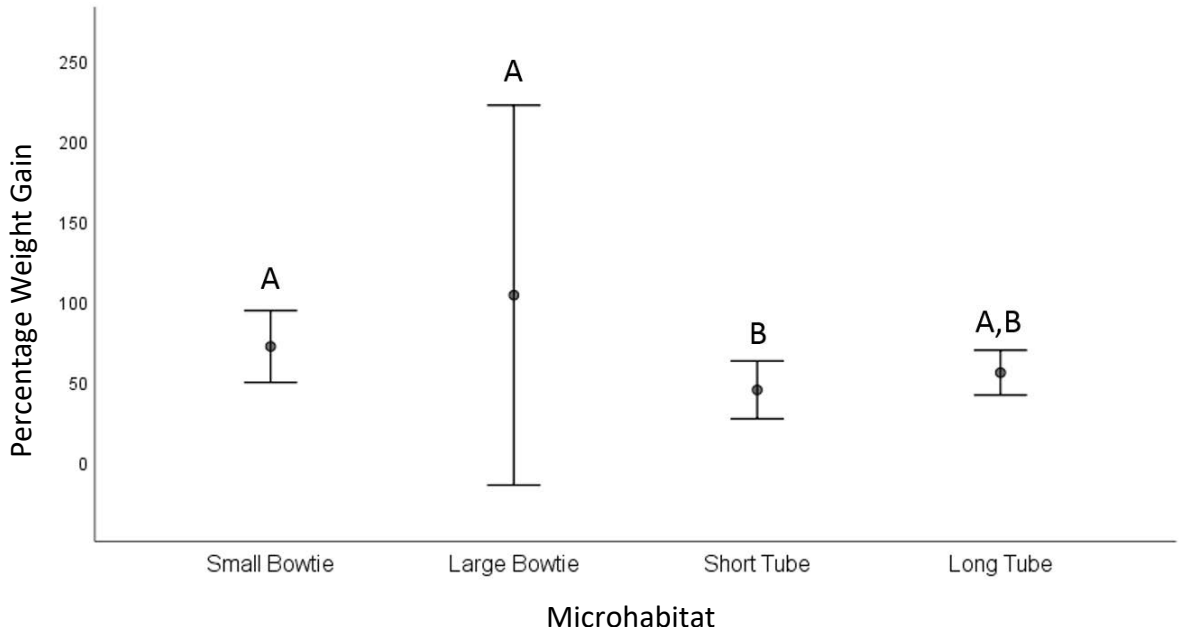

Figure 43. The percentage weight gain of J1 Cherax quadricarinatus exposed to one of four microhabitat treatments.
Error bars indicate the 95% Confidence Interval, corresponding letters indicate non-significant differences.

6.5.4 Discussion

The percentage survival was significantly lower for both sizes of the bowtie microhabitat than both sizes of the tube microhabitat (Figure 43), mean percentage weight gain was significantly higher for both sizes of the bowtie microhabitat than the short tube habitat (Figure 44). These results indicate that the bowtie microhabitat provided a habitat which offered less protection of the redclaw from each other than the tube microhabitat as their survival was much lower. Within the bowtie habitat, animals were able to see each other through the mesh and potentially cannibalise others whilst performing ecdysis. Furthermore, the mesh of the bowtie microhabitats was observed to 'catch' the on-grown *Artemia* sp. fed to the redclaw, which had the effect of collecting the food close to where the animals were positioned so they potentially did not need to leave the mesh to get access to food, hence the large weight gain, but

possibly also providing a vector for pathogens contributing to the lower survival. By contrast, the tube microhabitat treatments offered more protection to the animals from each other, as evidenced by the significantly higher survival percentage (Figure 43) than the bowtie microhabitat treatments. The tube microhabitats were of a width which only allowed one animal to use them, potentially animals could enter at the other end of the tube, but aggressive interactions were likely minimised due to the restricted space in the tubes. Although protective of the redclaw, the tube microhabitats required the animals to leave the protection of the tubes in order to approach food items. This may have had the effect of discouraging them to gather food through aggressive interactions if other animals were already out of the tubes, and hence the lower weight gain than the bowtie microhabitat treatments due to more difficult access to food.

The density treatments which varied between 11 redclaw per m^2 to 52 redclaw per m^2 involving 18 – 72 redclaw per tank (Table 6), had no significant effect on either percentage weight gain or percentage survival. The results were somewhat surprising as these densities appeared to be quite high in view of the literature and the expectation was to see a significant effect upon both weight and survivorship. For instance, an experiment was conducted with larger 1.3 g juveniles at stocking densities of 5, 6, 8, 11, and 20 crayfish per m^2 to attempt to achieve 25 g animals in 80 days and found that densities at or below 11 per m^2 could achieve this (Naranjo-Paramo *et al.* 2004). Low stocking densities of 3, 9, and 15 crayfish per m^2 achieved increased economic returns and yield with increased stocking size and density for advanced juveniles in studies by Naranjo-Paramo *et al.* (2004) and Jones and Ruscoe (2000). Neither Naranjo-Paramo *et al.* (2004) or Jones and Ruscoe (2000) used redclaw of the size and age used in our study and there were potentially confounding effects such as use of outdoor grow-out ponds for the experiments. However, it remains unclear as to why in our habitat and

density trials no effect of stocking density was seen on the relatively high densities and high range of different densities. We suspect that in order to see an effect of density on weight gain and survivorship, a much larger amount of habitat is required, as the survivorship in this study was low across all density and habitat treatments (8% large bowties – 37% long tubes).

Further studies to address the factors of habitat and stocking density upon growth and survivorship of nursery phase redclaw would benefit from using provisions of mesh one to two orders of magnitude larger. Larger areas of mesh may mitigate the high levels of mortality by providing further distance between redclaw and less concentration of *Artemia* sp. for potential pathogen spread. Similarly, distribution of feed closer to the ends of the tubes may increase the weight gain for redclaw using these as habitat. Despite the aforementioned caveats, mesh would appear to be more practical than tubes within a nursery phase, as the redclaw could easily out-grow the size of the tubes in an extended study. The mesh also has the benefit of capturing the food items, is easy to clean and use, and practical in terms of harvesting the redclaw. A broad range of stocking densities could be trialed alongside much larger microhabitat treatments to see if the microhabitat provision and therefore density can be extended to its upper limits whilst negating large-scale conspecific predation.

6.6 General Discussion

Although not forming the main body of the data chapters in this Thesis, the opportunity to perform three separate pilot or unreplicated studies has had the benefit of being able to test new ideas and set directions for future studies. In a nursery phase, nutrition is one of the key factors to enhancing the health, growth and condition of early instar redclaw. The biofloc tank pilot study shows promise for two reasons, firstly a simple and practical method was used to produce a floc indoors in tanks with or without

crayfish, and secondly there was a benefit to the redclaw in that the floc appeared to provide extra nutrition resulting in larger animals and higher survivorship. Biofloc for an indoor nursery phase has potential and should be pursued, perfected and used as a protocol. The second pilot study, initiated to explore whether an index for growth and survival similar to the lobster condition index for condition based on protein and lipids was applicable to redclaw, had mixed results. Percentage lipids and percentage protein showed some promise as an index of condition for survivorship, however none of the parameters correlated with indices for growth. As noted, there was considerable cannibalism involved in this study which may have confounded the results. Although not a clearly positive set of results, removing intraspecific predation from the proximate composition study may clarify the issue and provide information as to the potential as indices of condition, for both growth and survivorship. In a similar fashion, the effect of stocking density and shelter on the growth and survivorship of redclaw in a nursery phase appears to be confounded by the effect of low survivorship. One positive that did result from the study was that it appears that dressmaker's tulle, or similar material, would be a more practical choice for a nursery phase. The tube microhabitat had a higher survivorship but at the cost of less growth. The use of substantially-larger provisions of tulle may ameliorate the cannibalism experienced here and produce clearer results.

In conclusion, these pilot studies should be repeated and improved in terms of generating some replicated and clear results so that these future directions for a nursery phase can be included in a set of best practice protocols for a nursery phase for early instar *C. quadricarinatus*, redclaw. The biofloc study in tanks needs to be replicated and improved upon in terms of mitigating cannibalism to be able to discern the effect of the biofloc nutrition on the growth and survivorship in a nursery phase. Percentage

lipids and percentage protein showed some promise as an index of condition for survivorship, however none of the parameters correlated with indices for growth. Again here the results were potentially confounded by cannibalism, repetition of the experiment without cannibalism may clarify the usefulness of these measures as indices of both survivorship and growth. The density and habitat experiments showed promise in that the Tulle habitat promoted good growth, future experiments should focus on larger apportionments of this habitat and far higher densities to uncover the maximum density combined with the best combination of growth and survivorship. A refinement of the stocking density and habitat provision could then be used in both the biofloc in tanks and the proximate composition work to mitigate cannibalism, so that pending successful results, all three parameters could be added to a protocol for a nursery phase to on-grow J1 redclaw and potentially provide better growth, survivorship and predictability of yields at harvest.

6.7 Literature Cited

ALLAN GL AND MAGUIRE GB (1992). Effects of stocking density on production of *Penaeus monodon* Fabricius in model farming ponds. Aquaculture 107(1):49-66.

ANSON KJ AND ROUSE DB (1996). Evaluation of several commercial feeds and Crustacean Reference Diet for juvenile Australian Red Claw crayfish, *Cherax quadricarinatus*. Journal of Applied Aquaculture 6(1):65-76. doi: 10.1300/J028v06n01_06.

AVNIMELECH Y (2009). Biofloc technology. A practical guide book. The World Aquaculture Society, Baton Rouge.

BALLESTER ELC, MARZAROTTO SA, SILVA DE CASTRO C, FROZZA A, PASTORE I AND ABREU PC (2017). Productive performance of juvenile freshwater prawns *Macrobrachium rosenbergii* in biofloc system. Aquaculture Research 48(9):4748-4755.

BARKI A AND KARPLUS I (2004). Size rank and growth potential in Redclaw crayfish (*Cherax quadricarinatus*): are stunted juveniles suitable for grow-out? Aquaculture Research 35(6):559-567.

BARKI A, KARPLUS I, MANOR R, PARNES S, AFLALO E AND SAGI A (2006). Growth of redclaw crayfish (*Cherax quadricarinatus*) in a three-dimensional compartments system: Does a neighbor matter? Aquaculture 252(2-4):348-355. doi: 10.1016/j.aquaculture.2005.07.012.

BARKI A, LEVI T, SHREM A AND KARPLUS I (1997). Ration and spatial distribution of feed affect survival, growth, and competition in juvenile red-claw crayfish, *Cherax quadricarinatus*, reared in the laboratory. Aquaculture 148(2):169-177.

BITOMSKY J (2008). Scoping Report Redclaw Industry Development. Kleinhardt Business Consultants, Cairns.

BOWSER PR AND ROSEMARK R (1981). Mortalities of cultured lobsters, *Homarus*, associated with a molt death syndrome. Aquaculture 23(1-4):11-18.

CAMPAÑA-TORRES A, MARTINEZ-CORDOVA LR, VILLARREAL-COLMENARES H AND CIVERA-CEREDO R (2008). Carbohydrate and lipid digestibility of animal and vegetal ingredients and diets for the pre-adult redclaw crayfish, *Cherax quadricarinatus* (von Martens). Aquaculture Research 39(10):1115-1121. doi: 10.1111/j.1365-2109.2008.01980.x.

DANIELS WH, D'ABRAMO LR, FONDREN MW AND DURANT MD (1995). Effects of stocking density and feed on pond production characteristics and revenue of harvested freshwater prawns *Macrobrachium rosenbergii* stocked as size-graded juveniles. Journal of the World Aquaculture Society 26(1):38-47.

EKASARI J, SUPRAYUDI MA, WIYOTO W, HAZANAH RF, LENGGARA GS, SULISTIANI R, ALKAHFI M AND ZAIRIN JR M (2016). Biofloc technology application in African catfish fingerling production: the effects on the reproductive performance of broodstock and the quality of eggs and larvae. Aquaculture 464:349-356.

EL-SAYED AFM (2021). Use of biofloc technology in shrimp aquaculture: a comprehensive review, with emphasis on the last decade. Reviews in Aquaculture 13(1):676-705.

EMERENCIANO M, GAXIOLA G AND CUZON G (2013). Biofloc technology (BFT): a review for aquaculture application and animal food industry. Biomass now-cultivation and utilization:301-328.

FIGUEIREDO MSRB, KRICKER JA AND ANDERSON AJ (2001). Digestive Enzyme Activities in the Alimentary Tract of Redclaw Crayfish, *Cherax quadricarinatus* (Decapoda: Parastacidae). Journal of Crustacean Biology 21(2):334-344. doi: 10.1651/0278-0372(2001)021[0334:deaita]2.0.co;2.

GARCIA-GUERRERO M, HENDRICKX ME AND VILLARREAL H (2003). Description of the embryonic development of *Cherax quadricarinatus* (Von Martens, 1868) (Decapoda, Parastacidae), based on the staging method. Crustaceana V76(3):269-280.

GARZA DE YTA A (2009). Hatchery, nursery, nutrition and stock evaluation of redclaw crayfish *Cherax quadricarinatus*. Pp. 135. Auburn University.

GEDDES MC, SMALLRIDGE M AND CLARKE S (1993). The effect of stocking density, food type and shelters on survival and growth of the Australian freshwater crayfish, *Cherax destructor*, in experimental ponds. Freshwater Crayfish 9:57-69.

GUTIERREZ-WING MT AND MALONE RF (2006). Biological filters in aquaculture: trends and research directions for freshwater and marine applications. Aquacultural Engineering 34(3):163-171.

HARTNOLL R (1978). The determination of relative growth in Crustacea. Crustaceana 34(3):281-293.

JEFFS A, WILLMOTT M AND WELLS R (1999). The use of energy stores in the puerulus of the spiny lobster *Jasus edwardsii* across the continental shelf of New Zealand. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 123(4):351-357.

JONES C, VALVERDE C AND RIGG D (2018). Redclaw Crayfish Hatchery. Development of Mass Production Hatchery for *Cherax quadricarinatus*. The Association of Astacology's Twenty-Second International Symposium on Freshwater Crayfish. International Association of Astacology, Carnegie Museum of Natural History, Pittsburgh, Pensylvania, USA.

JONES CM (1989). The Biology and Aquaculture Potential of *Cherax quadricarinatus*. Final Report for Project "The Assessment of *Cherax quadricarinatus* as a Candidate for Aquaculture. Reserve Bank of Australia, Rural Credits Development Fund, Project QDPI/8860. Pp. 131. Department of Primary Industries, Queensland, Walkamin, Australia.

JONES CM (1990). Biology and aquaculture potential of the tropical Australian freshwater crayfish (*Cherax quadricarinatus*). Pp. 19. Abstracts, Eighth International Symposium of the International Association of Astacology, Baton Rouge, USA, April 22-26, 1990. International Association of Astacology, Baton Rouge.

JONES CM (1995a). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) I. Development of hatchery and nursery procedures. Aquaculture 138(1995):221-238.

JONES CM (1995b). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) II. Juvenile nutrition and habitat. Aquaculture 138(1995):239-245. doi: 10.1016/0044-8486(95)00069-0.

JONES CM (1995c). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) III. Managed pond production trials. Aquaculture 138(1995):247-255. doi: 10.1016/0044-8486(95)00067-4.

JONES CM AND RUSCOE I (1996a). Production Technology for Redclaw Crayfish (*Cherax quadricarinatus*). Final Report FRDC Project 92/119. Fisheries Research and Development Corporation, Canberra.

JONES CM AND RUSCOE IM (1996b). Evaluation of six diets fed to redclaw, *Cherax quadricarinatus* (von Martens), (Decapoda: Parastacidae) held in pond enclosures. Pp. 7-18 Production Technology for Redclaw Crayfish (*Cherax quadricarinatus*). Final Report, Project 92/119, Fisheries Research and Development Corporation. Department of Primary Industries, Queensland, Walkamin.

JONES CM AND RUSCOE IM (2000). Assessment of stocking size and density in the production of redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda: Parastacidae), cultured under earthen pond conditions. Aquaculture 189(1-2):63-71. doi: 10.1016/S0044-8486(00)00359-8.

JONES CM AND RUSCOE IM (2001). Assessment of five shelter types in the production of redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae) under earthen pond conditions. Journal of the World Aquaculture Society 32(1):41-52. doi: 10.1111/j.1749-7345.2001.tb00920.x.

JONES CM AND VALVERDE C (2020). Development of mass production hatchery technology for the redclaw crayfish, *Cherax quadricarinatus*. Freshwater Crayfish 25:1-6.

KANKAALA P, TAIPALE S, LI L AND JONES RI (2010). Diets of crustacean zooplankton, inferred from stable carbon and nitrogen isotope analyses, in lakes with varying allochthonous dissolved organic carbon content. Aquatic Ecology 44(4):781-795.

KARPLUS I AND BARKI A (2004). Social control of growth in the redclaw crayfish, *Cherax quadricarinatus*: testing the sensory modalities involved. Aquaculture 242(1-4):321-333. doi: 10.1016/j.aquaculture.2004.08.041.

KARPLUS I, BARKI A, LEVI T, HULATA G AND HARPAZ S (1995). Effect of kinship and shelters on growth and survival of juvenile Australian redclaw crayfish (*Cherax quadricarinatus*). Freshwater Crayfish 10:494-505.

LI J, LI J, LI W, SUN Y, LIU X, LIU M AND CHENG Y (2019). Juvenile *Procambarus clarkii* farmed using biofloc technology or commercial feed in zero-water exchange indoor tanks: A comparison of growth performance, enzyme activity and proximate composition. Aquaculture Research 50(7):1834-1843.

LOYA-JAVELLANA GN AND FIELDER DR (1997). Developmental trends in the mouthparts during growth from juvenile to adult of the tropical freshwater crayfish, *Cherax quadricarinatus* von Martens, 1868 (Decapoda: Parastacidae). Invertebrate reproduction & development 32(2):167-175. doi: 10.1080/07924259.1997.9672619.

MEADE ME AND WATTS SA (1995). Weight gain and survival of juvenile Australian crayfish *Cherax quadricarinatus* fed formulated feeds. Journal of the World Aquaculture Society 26(4):469-474.

MORRISSY NM, BIRD C AND CASSELLS G (1995). Density-dependent growth of cultured marron, *Cherax tenuimaus* (Smith 1912). Freshwater Crayfish 10:560-568.

NARANJO-PARAMO J, HERNANDEZ-LLAMAS A AND VILLARREAL H (2004). Effect of stocking density on growth, survival and yield of juvenile redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae) in gravel-lined commercial nursery ponds. Aquaculture 242(1-4):197-206.

PAVASOVIC A, RICHARDSON NA, MATHER PB AND ANDERSON AJ (2006). Influence of insoluble dietary cellulose on digestive enzyme activity, feed digestibility and survival in the red claw crayfish, *Cherax quadricarinatus* (von Martens). Aquaculture Research 37(1):25-32. doi: 10.1111/j.1365-2109.2005.01389.x.

PINTO GF AND ROUSE DB (1996). Growth and survival of the Australian red claw crayfish *Cherax quadricarinatus* at three densities in earthen ponds. Journal of the World Aquaculture Society 27(2):187-193.

QUEENSLAND GOVERNMENT (2016). Ross Lobegeiger Report to Farmers. Aquaculture production summary for Queensland 2015 - 16. Page 11, Queensland Government, Brisbane.

QUEENSLAND GOVERNMENT (2018). Ross Lobegeiger report to farmers. Aquaculture production summary for Queensland 2017-18. Page 8, Queensland Government, Brisbane.

QUEENSLAND GOVERNMENT (2020a). Ross Lobegeiger report to farmers. Aquaculture production summary for Queensland 2018-19 Page 11, Queensland Government, Brisbane.

QUEENSLAND GOVERNMENT (2020b). Water Monitoring Information Portol. Department of Natural Resources, Mines and Energy
Brisbane, Queensland.

RICE PR AND ARMITAGE KB (1974). The effect of photoperiod on oxygen consumption of the crayfish *Orconectes nais* (Faxon). Comparative Biochemistry and Physiology Part A: Physiology 47(1):261-270.

RIGG DP, COURTNEY RL, SEYMOUR JE AND JONES CM (2021a). Determining Suitable Thermal Regimes for Early Instar Redclaw Juveniles, *Cherax quadricarinatus* (von Martens, 1868) (Decapoda, Parastacidae), for a Proposed Nursery Phase. Freshwater Crayfish 26(1): 1-7.

RIGG DP, COURTNEY RL, SEYMOUR JE AND JONES CM (2021b). Evaluation of Four Practical Diets on the Growth and Survival of Juvenile Redclaw, *Cherax quadricarinatus* (von Martens, 1868). Freshwater Crayfish 26(1): 1-8.

RODGERS LJ, SAOUD PI AND ROUSE DB (2006). The effects of monosex culture and stocking density on survival, growth and yield of redclaw crayfish (*Cherax quadricarinatus*) in earthen ponds. Aquaculture 259(1):164-168.

SALONEN K AND HAMMAR T (1986). On the importance of dissolved organic matter in the nutrition of zooplankton in some lake waters. Oecologia 68(2):246-253.

SAOUD IP, GARZA DE YTA A AND GHANAWI J (2012). A review of nutritional biology and dietary requirements of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868). Aquaculture Nutrition 18(4):349-368. doi: 10.1111/j.1365-2095.2011.00925.x.

SAURABH S AND SAHOO P (2008). Major diseases and the defence mechanism in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). Proceedings of the National Academy of Sciences, India, Section B 78:103-121.

SHECHTER A, BERMAN A, SINGER A, FREIMAN A, GRINSTEIN M, EREZ J, AFLALO ED AND SAGI A (2008). Reciprocal changes in calcification of the gastrolith and cuticle during the molt cycle of the red claw crayfish *Cherax quadricarinatus*. The Biological Bulletin 214(2):122-134.

SHYNE ANAND PS, KUMAR S, KOHLI MPS, SUNDARAY JK, SINHA A, PAILAN GH AND DAM ROY S (2017). Dietary biofloc supplementation in black tiger shrimp, *Penaeus monodon*: effects on immunity, antioxidant and metabolic enzyme activities. Aquaculture Research 48(8):4512-4523.

THOMPSON K, MUZINIC L, CHRISTIAN T, WEBSTER C, MANOMAITIS L AND ROUSE D (2003). Lecithin requirements of juvenile Australian red claw crayfish *Cherax quadricarinatus*. Aquaculture Nutrition 9(4):223-230.

TIDWELL JH, COYLE S, WEBEL C AND EVANS J (1999). Effects and interactions of stocking density and added substrate on production and population structure of freshwater prawns *Macrobrachium rosenbergii*. Journal of the World Aquaculture Society 30(2):174-179.

ULLOA WALKER DA, MORALES SUAZO MC, AND EMERENCIANO MGC (2020). Biofloc technology: principles focused on potential species and the case study of Chilean river shrimp *Cryphiops caementarius*. Reviews in Aquaculture.

VIAU VE, OSTERA JM, TOLIVIA A, BALLESTER ELC, ABREU PC AND RODRÍGUEZ EM (2012). Contribution of biofilm to water quality, survival and growth of juveniles of the freshwater crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae). Aquaculture 324-325:70-78. doi: 10.1016/j.aquaculture.2011.10.009.

VIAU VE AND RODRÍGUEZ EM (2009). Substrate selection and effect of different substrates on survival and growth of juveniles of the freshwater crayfish *Cherax quadricarinatus* (von Martens 1868) (Decapoda, Parastacidae). Aquaculture International 18(5):717-724. doi: 10.1007/s10499-009-9292-0.

WHEATLEY MG (1999). Calcium homeostasis in Crustacea: the evolving role of branchial, renal, digestive and hypodermal epithelia. Journal of Experimental Zoology 283(7):620-640.

WHEATLEY MG AND AYERS J (1995). Scaling of calcium, inorganic contents, and organic contents to body mass during the molting cycle of the fresh-water crayfish *Procambarus clarkii* (Girard). Journal of Crustacean Biology 15(3):409-417.

XUE XM, ANDERSON AJ, RICHARDSON NA, XUE GP AND MATHER PB (1999). Characterisation of cellulase activity in the digestive system of the redclaw crayfish (*Cherax quadricarinatus*). Aquaculture 180(1999):373-386.

Chapter 7. General Discussion and Conclusions

7.1: Introduction

7.1.1 Project Background Redclaw Industry Perspective

In the late 1980's when Australian aquaculture was in its infancy and the demand for aquaculture products was starting to rise, the Queensland Department of Primary Industries (QDPI) was looking into prospective new species for aquaculture development. Initially the Western Australian marron, (*Cherax tenuimanius / cainii*) was thought to be a strong contender for aquaculture production in Queensland. A small industry was established, but marron were found largely unsuitable for aquaculture production in Queensland and the industry collapsed.

The idea of a freshwater crayfish aquaculture industry for Queensland was not abandoned however, and a more suitable candidate was sought. A species similar to marron, but native to Queensland, *Cherax quadricarinatus* was identified as such a candidate. Initial ad-hoc assessment by farmers identified a number of attributes of this species that suggested it might be suitable for aquaculture development. These included; low technology requirement and low-cost of production, a straightforward life cycle with much of the larval development occurring in the egg or conducted maternally, more than one breeding event per annum, growth to market size within a year, hardiness in terms of temperature and dissolved oxygen tolerance, premium taste, texture and presentation garnering a premium seafood price, and suitability to Queensland climatic conditions. At that time, *C. quadricarinatus*, commonly referred to as redclaw, was generally only known to local inhabitants of the remote and sparsely populated areas of the western flowing rivers and creeks of Cape York and the Gulf of Carpentaria drainage in Queensland. The ad-hoc farmer assessment that suggested the

suitability of the species, prompted the Queensland Government, through the Department of Primary Industries (DPI) to commission a scientific study into the feasibility of redclaw as a new aquaculture species (Jones 1989).

The QDPI biological assessment by Jones (1989) was conducted over the course of two years, and it built on the knowledge of the few farmers who had trialed redclaw since 1985 (Jones 1989). The biological assessment and the concurrent development of the industry confirmed redclaw to be an ideal species for aquaculture with no apparent obstacles to further development, and many positive aspects, such as:

- Established demand overseas for freshwater crayfish indicates potential in Australia (e.g. Europe, c. 7,000t per annum)
- Potential to develop a South-East Asian and Japanese market for live animals as they can be easily transported live
- Compares favourably to popular crustaceans in terms of size, colour and form, flavour, texture and flesh recovery rate
- Ideal physiological tolerance to temperature, dissolved oxygen and salinity
- Rapid growth to table-size within twelve months
- Inexpensive to feed due to a low protein requirement, non-aggressive, non-burrowing and can be held in high densities (5 - 10 m2)
- Ease of harvesting through flow-trapping, low technology requirement for broodstock and incubation of eggs, reasonably high fecundity, juveniles respond well to intensive nursery conditions
- Low handling mortality at all stages and a broad genetic pool extremely useful for selective breeding (Jones 1989).

In 1996 a second report was prepared by QDPI to outline the production technology required to establish a redclaw aquaculture farm, including a best-practice model outlining potential inputs and outputs (Jones and Ruscoe 1996a). In this study optimal farm characteristics were defined and economies of scale investigated for commercial viability, specifications for ponds, water and stock management protocols were all outlined, and this was all presented in a business-based framework (Jones and Ruscoe 1996a).

In January 2008 a scoping report was produced on behalf of QDPI to further accelerate the development of a substantial redclaw aquaculture industry (Bitomsky 2008). This report examined the state of the redclaw aquaculture industry at this time and assessed the business case for establishing a large-scale commercial aquaculture facility to fuel investment in the industry and grow the industry to the critical mass required to establish and supply markets both nationally and internationally. It found that the business case for an export scale redclaw industry was very strong and that the Queensland State Government should encourage investment, beginning with a large scale farm (Bitomsky 2008).

Despite these reports and many other endeavours by QDPI to encourage investment and involvement in the redclaw aquaculture industry, progress on industry expansion stalled. The production reported in 2018 - 19 was 44.9 t, worth A $1.2M, down 8.1% from 48.8 t and A $1.3M from the previous year (Queensland Government 2018, 2020a) and less than half the production of the highest production figure of 105 t in 2005-06 (Queensland Government 2016). The stagnation of industry development was puzzling, as the business case for redclaw aquaculture production remained sound.

The Queensland Crayfish Farmers Association consulted with the Rural Industries Research and Development Corporation (RIRDC, now AgriFutures) to

establish a research project to examine roadblocks to redclaw aquaculture investment and participation, “Identify Factors Influencing the Variability of Survivorship of Juvenile Redclaw Crayfish *Cherax quadricarinatus* in Aquaculture”. There were three sub-projects established within the framework of the AgriFutures project. The first was to address a bacterial problem regarding the eggs in the hatchery through the use of bacteriophage technology, a second sub-project examined adult handling mortality and underlying diseases, and the third sub-project (reported here in this thesis) examined methods to maximise the transition survival from hatchery to pond and to formulate best practice for the hatchery operator and farmer.

It was thought that intensification of production of the early life instars would be the way forward as it had been for other aquaculture enterprises like shrimp. Taking control of the production of seed stock through an intensive hatchery phase, followed by an intensive nursery phase to on-grow the seed stock, was thought to help combat one of the main barriers to investment which was highly variable harvests and therefore, income. Production of more robust seed stock which had been supplied with optimal conditions in terms of diet, thermal regime and protection from cannibalism was considered to be the way to increase subsequent survivorship in pond grow out, through to harvest and provide a less variable harvest and therefore more surety about income levels.

7.1.2 Knowledge Gaps

Previously, there was virtually nothing known about the requirements for early instar redclaw as production of seed stock was characteristically achieved through natural reproduction with minimal interference in the breeding and raising of early instars. The process involved selecting males and females for their size and positive characteristics and placing them in breeding ponds or tanks for spawning. Mature adults

and/or ovigerous females were stocked to juvenile production ponds that were managed to maximise zooplankton abundance as a food source for the offspring, and harvested after 3 months to generate advanced juveniles of around 5 to 10 g each for grow-out pond stocking (Jones 1995c, b, a). This traditional approach is not particularly efficient and prompted the development of hatchery technology (Jones *et al*. 2018) that generates much smaller J1-stage crayfish for stocking. Under this procedure, knowledge was lacking regarding the eggs and the hatched instars L1 and L2 where the animals are non-mobile, non-feeding and attached to the underside of the female's abdomen. Similarly, little was known of the requirements of the subsequent free-living J1, J2 and J3 instars.

The transition from pond-based juvenile production to hatchery production enabled more control of the production environment, but by the same token, exposed the lack of knowledge of the specific requirements for the initial instars. As the instars are distinct change points, phase transitions or critical moults (Hartnoll 1978), each instar may have its own requirements which would previously have been the domain of the maternal crayfish. The three instars from egg to L2 are non-exogenous feeding stages with all the required nutrition for growth and development stored in the egg yolk. As such, little can be done about enhancing nutrition in these stages except for the possibility that there is an uptake of dissolved organic matter when they hatch and moult. What is extremely important in these instars and the most important driver of the speed of growth and development is the most suitable thermal regime for each instar. The thermal regime may be the same for each of these instars or it may differ. The important thing is to make sure that the regime is warm enough to encourage a good development rate, but not too warm to force the development rate and exhaustion

of the energy store of egg yolk, which is essential to complete the energetically-expensive process of moulting (Rice and Armitage 1974).

7.1.3 Justification for this Study

This Thesis forms the first in-depth study of the requirements for a nursery phase, starting with the J1-stage redclaw, the first free-living, mobile and exogenously feeding instar. Furthermore, the combined work presented here on temperature, allometry and respirometry form the first study of its kind; a complete analysis of these factors for the crucial earliest six instars of redclaw. Specifically looking at the requirements of the first six instars allows for an examination of the optimum requirements in terms of nutrition and thermal regime from egg to instar J3, a period covering both the hatchery phase and a proposed nursery phase. This previously unknown information will allow a set of protocols to be developed that will enhance the survival and robustness of the redclaw seed stock to ensure they can be stocked into grow-out ponds in optimal condition to maximise production at harvest.

7.1.4 Planned Outputs – Protocols / Recommendations

The central theme around the series of experiments which were conducted and reported in this Thesis was to effectively trial and recommend a set of protocols for a prospective nursery phase. The nursery phase would follow on from the hatchery phase which is now well-established (Jones *et al.* 2018) and able to supply similarly-aged seed stock in commercial quantities. The proposed nursery phase will on-grow the seed stock to a more robust size with greater potential for survival and growth through to harvest in the grow-out pond phase of production.

Therefore, the planned output from this Thesis, combined with the pilot studies designed to map the future directions of research into redclaw, are to define the parameters in a nursery phase. The parameters include a practical diet recommendation

for a nursery phase which pertains to the instars J1 –J3, the allometric relationship between various lengths and weights in order to better outline the individual instars, and a long-term presumed suitable thermal range recommendation based on combined work in the area of survivorship and growth over a recommended presumed suitable thermal range for the time period involved in a nursery phase (*c*. 3 weeks), as well as detailed work examining respirometry and the performance of individual instars from the hatchery stages (egg to L2) through to the nursery phase stages (J1 –J3).

The parameters will be presented as a set of protocols designed for the redclaw aquaculture industry for a proposed nursery phase which can be adapted from the work presented here and used in industry. It is hoped that through the development of this set of protocols which mostly pertain to the nursery phase but also explore some information gaps in the hatchery phase such as metabolic performance over a wide range of temperatures for a short period of time, will assist in the development of the industry. As noted, the potential for the aquaculture of redclaw is still positive, especially in the light of an increasing world population requiring good quality seafood without causing further detrimental consequences for fish stocks worldwide which are under increasing pressure, and with the advent of the CoVid-19 pandemic in 2020, food security for individual nations has become of utmost importance due to travel and export restrictions. Redclaw has the potential to provide protein to many areas worldwide from the tropics to the sub-tropics, with a simple life cycle and relatively few cost inputs. Intensification of the aquaculture industry including taking control of the production of seed stock in a hatchery phase, plus an additional nursery phase to add strength and vigour for grow-out to market size, are the next steps to making this a reality.

7.2: General Discussion and Conclusions from Data Chapters

7.2.1 Chapter 2: Evaluation of Four Practical Diets on the Growth and Survival of Juvenile Redclaw, *Cherax quadricarinatus* (von Martens, 1868)

Chapter 2 formed the first of the four main data chapters that addressed specific components of a proposed nursery phase and looked at whether one of the four chosen practical diets could be a suitable candidate for a nursery phase. The aim was to evaluate whether one of these diets would assist in the main goals of a nursery phase, to promote high survivorship and acceptable growth rates through this period to produce more advanced and robust juveniles for release into grow-out ponds.

The diets trialed in this study were chosen for their practicality, availability, component nutrition and cost effectiveness. The experiment ran for two weeks and the result was that redclaw which were at J1 instar at the start of the experiment fed either *Artemia* sp. or Frippak experienced a larger weight increase than the J1s fed bloodworms or the CSIRO diet. In terms of biomass the *Artemia* sp. treatment animals were the highest amongst the four treatments.

There was a mortality spike between days six and nine for the Frippak and CSIRO treatments, attributed to the accessibility of the feed, rather than any nutritional deficiency. Both of these treatments became bound to the bottom of the experimental apparatus, the inability to access the feed may have left these animals nutritionally-compromised and without the energetic reserves to complete ecdysis, leading to their death via ‘molt-death syndrome’ (Bowser and Rosemark 1981; Meade and Watts 1995; Anson and Rouse 1996; Thompson *et al*. 2003) or ‘exuvia entrapment disease’ (Saurabh and Sahoo 2008).

Both the *Artemia* sp. and bloodworms treatments showed the highest survival through the study, the highest weight gain was attributed to *Artemia* sp. and Frippak diets. It has been suggested that redclaw have a higher protein requirement as juveniles than adults (Saoud *et al*. 2012), an ontogenetic dietary shift, whereby juveniles have a much higher protein requirement than adults. The results here support this as the higher protein content feeds, bloodworms and *Artemia* sp., produced the highest combination of survivorship and growth, however this was not conclusive as the physical form of the manufactured diets appeared to have an adverse impact that may have masked the adequacy of the nutrient content. Overall, the most suitable diet for a nursery phase of the four diets trialed in this instance, based on the combination of survival, weight gain and biomass, was the *Artemia* sp. diet.

Future directions for studies into diet for a nursery phase for redclaw produced in a hatchery system would suit using *Artemia* sp. as a reference diet to compare against, however a high-specification compound diet designed with such form as to conform to redclaw morphology and behaviour warrants consideration. Whether such a nursery phase would be conducted in a tank system or outdoor pond is also worth consideration.

7.2.2 Chapter 3: Determining Suitable Thermal Regimes for Early Instar Redclaw Craylings Cherax quadricarinatus (von Martens, 1868) (Decapoda, Parastacidae) for a Proposed Nursery Phase

Chapter 3, the second data chapter, examined thermal regimes for J1-aged redclaw over the period proposed for a nursery phase, and at temperatures known to be well within the range experienced by redclaw in grow-out ponds. Temperatures in grow-out ponds in southern Queensland at the southern extreme of redclaw farming in Queensland can dip as low as 18°C for up to five weeks annually near Gympie and in their natural range redclaw can experience high temperatures of 28 - 31°C in the Walsh

River from January to February (Queensland Government 2020b). The aim was not to test the long-term survivability of the extremes of the temperature treatments, but rather to find the most suitable temperature to provide optimum growth and survivorship over this period of twenty-two days after J1 instar.

There was found to be a significant effect of temperature on the survivorship of J1 redclaw over the course of the experiment, high temperature treatments were associated with high levels of mortality, and low temperature treatments experienced very low mortalities, in fact zero mortalities in two treatment groups. Temperature treatments 18°C, 19.5°C and 22°C had percentage survival of 100%, 98% and 100% respectively. The higher temperature treatments between 25°C and 32°C had a varying survival between 0% and 6%. The majority of mortalities in the higher temperature treatments between 25°C and 30°C occurred between days six and eleven in the experiment which correlated with the initiating of moulting. Weight gain of redclaw was significantly higher with increasing temperature treatments between 18°C and 22°C, and weights at the end suggested that they had successfully completed one moult from J1 to J2.

The recommendation in this instance is that J1-aged redclaw held in tanks at a constant temperature over the course of a nursery phase do best in terms of survivorship and growth between 18°C and 22°C, and for optimum growth 22°C. Future directions for investigation into a nursery phase should look to couple the results from this long-term trial with short-term respirometry experiments at different temperatures within the range studied here, to look into the metabolic rate side of the thermal question.

7.2.3 Chapter 4: Morphology and Weight-Length Relationships for the First Six Instars of Cherax quadricarinatus (von Martens, 1868)

Prior to the development of hatchery technology when juvenile production was pond-based, there was little direct management applied by redclaw aquaculture farmers to the early instars from egg through to the J1 free-living instar. This developmental phase occurred out of sight, in the juvenile production ponds, affording the farmer no opportunity to examine or learn about these stages. As such, little was known about the early life history of the species until a study by Jones (1989) described the biology and potential for aquaculture. An excellent account of embryonic development from egg to J1 was given by Garcia-Guerrero *et al.* (2003), however now that a nursery phase is proposed, the knowledge of production requirements of the early instars is required.

A certain amount of confusion in naming the particular instars from egg to J3 has been evident in the literature over the years, partly because of uncoordinated research efforts globally, but also due to collective terms such as “juvenile” or “crayling” grouping a number of instars. As such a new naming system is proposed here, providing continuity and separation of naming of the larval non-exogenous feeding instars from the free-living, motile exogenous-feeding instars. Based on gross morphology and allometric relationships, the naming system for the first six instars proceeds as: Egg, L1, L2, J1, J2 and J3. The size of each instar was defined through linear regression and descriptions and photographs were provided to aid identification. There was a significant relationship established between wet weight and ocular carapace length, which increased with each successive instar for five of the six instars, lyophilized (dry) weight was not significantly different for instars from egg to J1 inclusive. An increase in ocular carapace length and wet weight but not lyophilized (dry) weight across instars L1, L2 and J1 indicates nutrition other than that supplied by

the yolk. Uptake of dissolved organic matter (DOM) across the integument gills or at ecdysis may be a possible way of making up this energetic deficiency and may form a way to supply another food source to enhance the condition of redclaw in a nursery phase and beyond to harvest.

7.2.4 Chapter 5: Thermal Tolerance of the Early Life Instars of Redclaw Crayfish Cherax quadricarinatus (von Martens, 1868).

Thermoconformers such as redclaw rely entirely on environmental thermal regimes for thermoregulation, therefore within a hatchery phase and nursery phase, water temperature is important for the redclaw in order for them to be able to consume and convert resources such as food into growth, reproduction and survival. Each instar may have a different presumed suitable thermal range to maximise growth and survivorship. This information does not currently exist for the instars continuously from egg through to instars J2 and J3 which would be involved in a nursery phase. A short-term method of assessing presumed suitable thermal range(s) is to measure oxygen consumption rates at different temperatures as analogous to metabolic rate. Experiments were performed to examine oxygen consumption rate over a range of temperatures for redclaw from egg through five moults to J3 to discover the presumed suitable thermal range which may be suitable for nursery and hatchery phases for these instars.

Experiments were conducted at ten temperatures, 16°C to 39°C, J3 instar were also trialed at 41°C, all with 20 replicates each. Suitable temperature range for each life instar was ascertained via identification of the section of the oxygen consumption curve which was moderately high in oxygen consumption, but not unsustainable in the long term, that is, the middle section of the graph where individual temperature treatments

were not statistically different from each other. Each interval between temperatures was also calculated as a slope via linear regression, significantly different slopes were denoted as change points in rates of oxygen consumption. Within each band of non-significantly different slopes each was compared to the first via pairwise ANCOVA and combined to define a predicted suitable long-term temperature range for each instar.

The predicted long-term temperature range for eggs from this data is 22°C to 28°C, L1 instar redclaw showed a larger range of 22°C to 31°C, L2 was from 20°C to 26°C and J1 instar also showed a range of 20°C to 26°C. J2 instar redclaw showed the same range as eggs, 22°C to 28°C, J3 instar produced the same range as J1 20°C to 26°C.

These ranges indicate high thermal plasticity showing redclaw as a generalist thermo-conformer. Other studies have shown optimal egg incubation at 22°C to 25°C (García-Guerrero *et al*. 2013) and natural populations of redclaw begin breeding at 21°C to 22°C (Sammy 1988; Jones 1990). The start point for the presumed suitable thermal ranges for all the instars here occurs at 20°C or 22°C, close to the optimal long-term thermal regimes recommended from other studies (Sammy 1988; Jones 1990; García-Guerrero *et al*. 2013). Rigg *et al*. 2021a, is the only study to examine temperature over a longer period of time (22 days) for instars J1, J2, J3 and found an optimal temperature of 22°C, here the presumed suitable thermal range start points are 20°C, 22°C and 20°C respectively. The rationale for the redclaw farmer is that the holding temperature for each of the first instars should be far lower than that presently practiced to optimise survival and growth across a hatchery and nursery phase.

The lecithotrophic non-exogenous feeding instars from egg to L2 rely on stored egg yolk for energy, therefore a low temperature for these instars may be advantageous to slow growth and spare yolk to successfully complete moults, allowing for

survivorship and growth through to harvest. Instar L1 has the broadest short-term presumed suitable thermal range of 9 degrees (22°C to 31°C) a large change from the presumed suitable thermal range for egg, 6 degrees from 22°C to 28°C, and L2 also 6 degrees but from 20°C to 26°C (Figure 25). This broadening in presumed suitable thermal range may reflect an ontogenetic or developmental change as L1 instar animals are attached to the maternal female, and the female may be searching varying microhabitats with a broad range of temperatures in the search for food, after carrying the eggs for a long period. The broader tolerance of temperatures at L1 may allow for this behaviour.

Current industry practice for redclaw hatcheries is to supply juvenile redclaw for pond stocking at J1 instar, however, if a proportion of those animals are L2, they are less equipped to withstand the physiological stress of transfer to grow-out facilities and may explain some of the variability in survival. High temperatures in grow-out ponds would be less suitable for introduction of J1 and J3 as opposed to J2 instar as J2 instar are able to withstand a higher temperature regime (Figure 25), a nursery phase to take the redclaw through to J2 infers a thermal tolerance benefit; however for maximum survival, release of any animals from J1 to J3 should be done when the grow-out pond temperature regime is lower at the start of Summer. Redclaw will cease to use food for somatic growth above certain temperatures, or achieve lower growth efficiencies (Meade *et al*. 2002), therefore in the pessimum range feed intake is used for maintenance alone (Sokolova *et al*. 2012). Feed being one of the largest costs in growing redclaw infers a lack of growth in a nursery phase worthless to the redclaw farmer.

This study is the first exploration of thermal tolerance through the use of oxygen consumption data as analogous to metabolic rate for the first six instars of *C.*

quadricarinatus at the range of temperatures expected in the grow-out pond environment on redclaw farms in the state of Queensland, Australia. This work can now form the basis for an energy budget, the next logical step in the intensification of redclaw aquaculture.

7.2.5 Chapter 6: Additional Pilot Studies and Investigations into Factors Pertaining to a Nursery Phase for Redclaw Crayfish *Cherax quadricarinatus*

7.2.5a Biofloc Production in Tanks as a Supplementary Nursery Phase Food Source

The large advantage in terms of nutrition which is conferred to J1 animals released for grow-out into ponds on redclaw farms comes from the natural productivity encouraged and enhanced by the aquaculture farmer in the form of zooplankton and phytoplankton (Ulloa Walker *et al*. 2020). Due to the fact that for ease of control and convenience, a nursery phase may be conducted in tanks in an indoor situation, the J1 redclaw need to be provided with equivalent or better nutrition than that of the natural productivity from grow-out ponds. An unreplicated study was initiated to discover whether blooms of biofloc, conglomerates of microbes, algae and protozoa along with dead organic particle and detritus (Avnimelech 2009) and nitrying bacteria, could be produced in a small scale in the laboratory as a food source for early instar redclaw. The second part attempted to discover whether the floc which was produced conferred a benefit to J1 redclaw crayfish in terms of survival and growth.

In the first part of the pilot study, biofloc production was attempted using methods adapted by Ballester *et al*. (2017) and Ekasari *et al*. (2016) with crayfish in one treatment, the other treatment without redclaw. Following this initial part of the experiment, the biofloc treatment without redclaw was used to seed one treatment called

biofloc, the other treatment was named clearwater, without biofloc. Both treatments had equal numbers and mass of redclaw J1 instar.

The result was that in the first part of the pilot study, both treatments managed to produce biofloc at day 14. Total ammonia nitrogen peaked three days apart, nitrite levels began three days apart, and nitrate levels peaked three days apart with the redclaw treatment occurring first in all instances. The cycling of the ammonia through nitrite and nitrate showed evidence of the presence of nitrobacter.

The second part of the study showed that the use of a biofloc starter culture to stimulate further biofloc production conferred advantages to the J1 redclaw over the clearwater treatment. The mean weight of individuals, the total wet weight at the end, the number of survivors, the mean percentage weight change and the percentage of original biomass, were all larger for the biofloc treatment.

This pilot study showed great potential for biofloc use in a nursery phase for J1 redclaw in tanks in an indoor situation, and with replication and refinement including tackling a problem with cannibalism, may be a way to enhance nutrition in a closed recirculating aquaculture system. Tank biofloc may also be a way to test the uptake of dissolved organic matter in all the post-hatch instars involved in a hatchery-nursery system, especially the non-exogenous feeding instars L1 and L2. Further advantages in utilising biofloc include water quality improvements, recycling of nutrients, low cost to establish and maintain, and the ability to operate closed recirculting aquaculture systems with less water demand and greatly reduced release of eutrophic water to the outside environment.

7.2.5b Protein / Lipid Composition Analysis as a Proxy for Condition in a Nursery Phase for Redclaw

An experiment was initiated to trial four feeds for J1 redclaw in a diet trial held over six weeks. The feeds and methods were the same as Rigg *et al*. 2021b, i.e. *Artemia* sp. bloodworms, a CSIRO formulation and the larval prawn feed Frippak, however the redclaw were kept en masse rather than individually. An opportunity arose to subsample redclaw at the four-week mark and the six-week mark and also for the remaining animals alive at the end. Lyophilized animals from these sampling events were analysed for protein and lipids weights as per the methods of Jeffs *et al.* (1999) to see if the different diets produced different proximate composition of the redclaw juveniles, if these varied at different times, and if these parameters were a good indicator of condition when compared to growth and survivorship data.

At week four the redclaw fed the Frippak treatment were significantly longer in ocular carapace length, significantly heavier in lyophilized weight and significantly higher in lipid weight and protein weight than the other diet treatments, however there was a similar percentage lipid of dry weight for all redclaw. The week six data showed the redclaw fed the Frippak diet treatment were significantly longer in ocular carapace length, significantly heavier in dry lyophilized weight, significantly heavier in wet weight, significantly heavier in lipid weight and significantly heavier in protein weight than redclaw fed the *Artemia* sp. bloodworms or CSIRO diets. The percentage protein of the lyophilized weight was significantly higher for the CSIRO diet for all diets but the Bloodworms.

The difference between the four-week and six-week data showed that the Frippak treatment was highest for ocular carapace length, lyophilized weight, wet weight, lipid weight and protein weight, the largest change in percentage lipids was the *Artemia* sp. diet treatment and CSIRO treatment redclaw had the least negative decrease in the percentage protein of lyophilized weight. The highest biomass for redclaw

remaining alive at the end of the experiment was the CSIRO diet treatment and the significantly highest wet weight was for the Frippak diet treatment, the other three diet treatments were not significantly different from each other. The surviving redclaw at the conclusion of the experiment varied from 42 animals, 16.7% survival (CSIRO diet) to 14 animals, 5.5% survival (Frippak diet).

The initial aim with this study was to ascertain whether any of the measured indices for protein and lipid composition would be a good indicator for condition; condition being the combination of growth and survivorship. In terms of growth, the metrics used to assess this were ocular carapace length, lyophilized weight and wet weight. Survivorship was measured as the percentage of the original animals remaining at the conclusion of the study at six weeks. The Frippak treatment redclaw were longest in carapace length and heaviest in lyophilized weight at weeks four and six, and also wet weight at week six, and largest in all these parameters as the change between weeks four and six, so it follows that they also had the significantly highest lipid weight and protein weight as well. Therefore, the Frippak showed the best result in terms of growth, but this was not reflected in the proportional measurements of percentage lipids content or percentage protein content, so they were not good indices for the condition parameter growth. The survivorship at the conclusion of the study was poor, and when analysing the protein and lipids data for each diet treatment, the only metric which showed any apparent correlation between the highest survival at the end for CSIRO treatment was percentage protein of dry weight at week six, this was however confounded by the fact that it does not differ significantly from the data for bloodworms.

As this was an opportunistic pilot study to ascertain the efficacy of using various measures of lipids and protein composition as indices for condition, the information generated is useful but not entirely a clear result. The factor which clouds the results is

potentially cannibalism, with low survivorship for all diet treatment redclaw, but most importantly the Frippak treatment which grew the largest, but experienced 94.5% mortality. With such a large degree of cannibalism it becomes difficult to ascertain where the redclaw received their nutrition in what was a diet trial. It would appear that speculatively there may be some promise in using the measurement of percentage lipids and proteins of lyophilized weight as indices for condition, but an experiment where cannibalism is excluded is required to make the results clearer.

7.2.5c Effect of Stocking Density and Shelter on Culture of Juvenile Redclaw

Despite their generally non-aggressive nature, redclaw can be cannibalistic when exposed to vulnerable conspecifics that are soft after moulting. This is particularly a problem when the moulting is frequent as it would be in a nursery phase. The level of stocking density can also be a factor associated with cannibalism due to physical proximity which can also have some density-dependent effects including reduction of growth and lower yields at harvest. Provision of habitat or shelter may contribute to increasing growth rates and may safely shield the animals from each other, negating aggressive interactions and cannibalism.

In the grow-out phase on redclaw aquaculture farms, artificial shelter is provided in the ponds for habitat, often pipe structures for larger redclaw and bundles of netting for smaller crayfish. A nursery phase needs to minimise the effect of cannibalism and high density effects to be successful, both in terms of survivorship and growth. Based on the habitat used in aquaculture farming of redclaw a pilot study sought to emulate artificial shelter at a scale more suitable to the size of the nursery phase redclaw. To replicate the pipe structures in miniature, twelve drinking straws were held inside a 2.54 cm (1”) diameter plastic pipe. To replicate the netting,

dressmaker's tulle was used, tied into a "bowtie"-type formation to provide three-dimensional space. Both types of habitat were trialed in two sizes, and densities between 11 and 52 redclaw m^2. Data were collected and analysed for treatment effects on total percentage weight gain and survival percentage.

The results indicated that the density treatments across the two different habitat types and the two sizes were not significantly different. The mean percentage survival was significantly higher for both lengths of tube habitat; the percentage weight gain was generally greater for both sizes of bowtie habitat. The bowtie habitat provided less protection from cannibalism than the tube habitat and therefore lower survivorship, perhaps because of the nature of the tulle mesh that enabled individuals to see each other and potentially prey on conspecifics that had just moulted. An advantage to the mesh however was that the food source (*Artemia* sp.) were caught in the mesh, allowing redclaw to gain nutrition without having to leave the shelter as evidenced by the percentage weight gain. The tube habitat performed well in terms of survivorship due to the restricted space in each allowing only one animal to take up residence and therefore to mitigate aggressive interactions. The lower percentage weight gain may be due to the discouraging requirement to leave the safety of the tubes to gather nutrition. There were no significant effects of density treatment on the results, which was surprising given that they were generally much higher densities than those seen in the relevant literature. An explanation may lie in the survivorship experienced in this study, (8% large bowties – 37% long tubes) neither group performed well or at the level required for a successful nursery phase. It is proposed that much larger provisions of both types of habitat be trialed to ascertain the size and type which would provide enough growth and survivorship to be practical in a nursery phase.

7.3: Recommended Protocols for a Redclaw Nursery Phase

1. Diet. Until a manufactured diet can be formulated which supports superior survivorship and growth in a nursery phase, *Artemia* sp. is the recommended nursery diet. *Artemia* sp. should also be used as the control or reference diet when assessing other candidate diets.

2. Temperature. Within a nursery phase, starting with J1 redclaw, the highest growth and survivorship was found at 22°C. The next best temperature tested was 25°C and between these two temperatures the survivorship dropped from 100% to 6%. Ascertaining the specific temperature between 22°C and 25°C at which mortalities begin to occur, may enable more rapid growth for temperatures higher than 22°C. Based on current data the best temperature is 22°C for growing from J1 to J3.

3. Allometry. The new designation of instars; Egg, L1, L2, J1, J2 and J3 clarifies the nomenclature of the initial life stages. Descriptions of sizes and weights should aid in identifying the older instars J2 and J3. Wet weight and ocular carapace length can be determined from each other.

4. Oxygen Consumption. The instars from egg through to J3 show some variation in thermal regime, but the rate of metabolism starting at 22°C for Egg, L1 and J2 and 20°C for L2, J1 and J3 suggests cooler rearing temperatures may be beneficial for early life stages, and a varying thermal regime that suits each life stage may maximise growth and survivorship.

5. Nursery Duration. In order to minimise on-costs for the farmer or hatchery / nursery operator, the duration of a nursery phase should be long enough to add positive benefits to the redclaw, but not too long to be uneconomical. To achieve two moults (J1 to J3) over a period of three weeks appears to be acceptable, dependent upon holding temperature.

6. Biofloc for Tanks. A basic recipe for biofloc production in indoors in tanks has been described and biofloc appears to provide beneficial conditions for the culture of redclaw. Additional research is required to optimise biofloc for redclaw.

7. Indices of condition: The proximate composition of lipid and protein in the body of juvenile redclaw has potential as a measure of physical condition that may be useful as a predictor of future condition.

8. Habitat and Density. Provision of artificial shelter appears to be important for the juvenile stages of redclaw to minimize cannibalism and support rapid growth. In combination with stocking density, these variables require further research to identify optimal practice.

7.4: Future Directions

7.4.1 Nursery Phase, Tanks or Ponds

In order for a nursery phase to be able to be implemented for the redclaw aquaculture industry, there is a need to fully assess the relative merits of tank and pond-based approaches. While tanks provide opportunity for intensive management of variables, ponds provide natural production of zooplankton and phytoplankton, proven to afford benefits of shade and nutrients. Perhaps a system similar to that trialed by Jones and Ruscoe (1996b) where small cages were fixed to the bottom of the grow-out pond, could be used. Cage mesh would need to be of a small enough size to hold J1 redclaw, probably in the order of 1 mm, that in turn may lead to biofouling and negative consequences. Tanks provide the advantage of temperature and water quality control, and in combination with biofloc production, provide a pond-equivalent to natural food production.

7.4.2: Formulated Diets Specific to Ontogenetic Change Points

Diet trials need to be performed to establish a manufactured diet for convenience, storage and cost, which should be tested against *Artemia* sp. - the best candidate of the four tested this study. The manufactured diet should have the same proximate composition, protein to energy ratio and digestibility as the *Artemia* sp. as well as an attractant such as shrimp hydrolysate or indeed small amounts of *Artemia* sp. to mimic all the positive aspects of feeding whole animal *Artemia* sp. A combination of the diets tested here, and a new manufactured diet could also be trialled for efficacy.

The relative levels of protein, carbohydrate and lipids for J1 redclaw have been established, but the acknowledged ontogenetic shift in diet towards a lower protein, more plant-based diet has not. The timing of this shift in preference away from a high

protein diet is important information and may involve more than one change. The raptorial dentition of the J1 redclaw confer an ability to capture zooplankton and minute benthic invertebrates (Loya-Javellana and Fielder 1997). One possible method of tracking the ontogenetic diet shift would be to follow changes in dentition from the J1 instar through to harvest size. This could be paired with food preference studies to ascertain the change points and the preferred diet at each point. The benefit to the redclaw aquaculture farmer in this instance would be to maximise feed composition at crucial change points, enhancing the health and growth of the redclaw, and potentially saving money on inappropriate levels of expensive protein components and maximising returns in terms of the harvest of large healthy crayfish.

7.4.3: Dissolved Organic Matter

The non-exogenous feeding stages of redclaw, i.e. Eggs, L1 and L2 rely on stored egg yolk to facilitate development and for metabolic needs and processes. Across these instars we found that the lyophilized weight (dry weight) does not change significantly, however the parameters of ocular carapace length and wet weight do increase across these instars (Rigg *et al*. 2021c). It has been noted for another freshwater crayfish species that there is an ability to absorb water and dissolved nutrients from the surrounding environment via branchial uptake (Wheatly and Ayers 1995), and it has been proposed that this may be the mechanism through which redclaw are receiving the extra nutrition extraneous to the use of stored yolk. This is an area ripe for exploration and a potential route for enhancing the growth and performance of the first three instars of redclaw. Firstly, discovering the mechanics of the way in which the redclaw are ingesting dissolved organic matter from the water column would be extremely useful. This information combined with trials to establish the nature of what organic matter is being absorbed could be a possible way to add these organic suspended materials to the

water column to assist the early development instars. Enhancing the condition of redclaw at the earliest stages of life should presumably flow on to health and survival advantages in later instars.

7.4.4: Energy Budget

Some of the most difficult data to obtain in the biology of aquaculture species is the resting metabolic rate at different temperatures. This information now exists for the first six instars of redclaw: Chapter 5, "Thermal tolerance of the early life instars of redclaw crayfish *Cherax quadricarinatus* (von Martens, 1868)".

In combination with diet studies where bomb calorimetry is used to establish the calorific content of the particular feed, all the information is in place to establish an energy budget for the first crucial six instars of redclaw. Having an energy budget would establish the amount of a particular feed that each instar would, on average, require to maximise use of feed provided and save on unnecessary excess feed. This may pass on to the redclaw aquaculture farmer considerable benefits in terms of cost savings. Following this, the next priority is the resting metabolic rate for life stages from J3 through to harvest size or growing time, at the range of temperatures experienced in the grow-out ponds. Knowledge gained on the ontogenetic changes in diet and the requirements in terms of feed at each change point would be necessary to hone the energy budget, but once established, create a 'menu' for the entire life cycle of redclaw crayfish. The benefits that this information could confer to the aquaculture industry for redclaw may assist in taking the industry to its much-anticipated potential.

7.4.5 Conclusion

Based on the findings of these studies a nursery phase is certainly warranted for the development of the redclaw aquaculture industry. The nursery phase would only require minor alterations in diet, culture temperature, and holding time before pond stocking, and may vastly improve the robustness, growth and survivorship of seedstock released into grow-out ponds. Potentially improved yields and more predictable outputs would translate into more a more stable investment climate and earnings for the farmer, returning to the promise of a thriving redclaw aquaculture industry.

7.5: Literature Cited

ANSON KJ AND ROUSE DB (1996). Evaluation of several commercial feeds and Crustacean Reference Diet for juvenile Australian Red Claw crayfish, *Cherax quadricarinatus*. Journal of Applied Aquaculture 6(1):65-76. doi: 10.1300/J028v06n01_06.

AVNIMELECH Y (2009). Biofloc technology. A practical guide book. The World Aquaculture Society, Baton Rouge.

BALLESTER ELC, MARZAROTTO SA, SILVA DE CASTRO C, FROZZA A, PASTORE I AND ABREU PC (2017). Productive performance of juvenile freshwater prawns *Macrobrachium rosenbergii* in biofloc system. Aquaculture Research 48(9):4748-4755.

BITOMSKY J (2008). Scoping Report Redclaw Industry Development. Kleinhardt Business Consultants, Cairns.

BOWSER PR AND ROSEMARK R (1981). Mortalities of cultured lobsters, *Homarus*, associated with a molt death syndrome. Aquaculture 23(1-4):11-18.

EKASARI J, SUPRAYUDI MA, WIYOTO W, HAZANAH RF, LENGGARA GS, SULISTIANI R, ALKAHFI M AND ZAIRIN JR M (2016). Biofloc technology application in African catfish fingerling production: the effects on the reproductive performance of broodstock and the quality of eggs and larvae. Aquaculture 464:349-356.

GARCIA-GUERRERO M, HENDRICKX ME AND VILLARREAL H (2003). Description of the embryonic development of *Cherax quadricarinatus* (Von Martens, 1868) (Decapoda, Parastacidae), based on the staging method. Crustaceana V76(3):269-280.

HARTNOLL R (1978). The determination of relative growth in Crustacea. Crustaceana 34(3):281-293.

JEFFS A, WILLMOTT M AND WELLS R (1999). The use of energy stores in the puerulus of the spiny lobster *Jasus edwardsii* across the continental shelf of New Zealand. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 123(4):351-357.

JONES C, VALVERDE C AND RIGG D (2018). Redclaw Crayfish Hatchery. Development of Mass Production Hatchery for *Cherax quadricarinatus*. The Association of Astacology's Twenty-Second International Symposium on Freshwater Crayfish. International Association of Astacology, Carnegie Museum of Natural History, Pittsburgh, Pensylvania, USA.

JONES CM (1989). The Biology and Aquaculture Potential of *Cherax quadricarinatus*. Final Report for Project "The Assessment of *Cherax quadricarinatus* as a Candidate for Aquaculture. Reserve Bank of Australia, Rural Credits Development Fund, Project QDPI/8860. Pp. 131. Department of Primary Industries, Queensland, Walkamin, Australia.

JONES CM (1995a). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) I. Development of hatchery and nursery procedures. Aquaculture 138(1995):221-238.

JONES CM (1995b). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) II. Juvenile nutrition and habitat. Aquaculture 138(1995):239-245. doi: 10.1016/0044-8486(95)00069-0.

JONES CM (1995c). Production of juvenile redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda, Parastacidae) III. Managed pond production trials. Aquaculture 138(1995):247-255. doi: 10.1016/0044-8486(95)00067-4.

JONES CM AND RUSCOE I (1996a). Production Technology for Redclaw Crayfish (*Cherax quadricarinatus*). Final Report FRDC Project 92/119. Fisheries Research and Development Corporation, Canberra.

JONES CM AND RUSCOE IM (1996b). Evaluation of six diets fed to redclaw, *Cherax quadricarinatus* (von Martens), (Decapoda: Parastacidae) held in pond enclosures. Pp. 7-18 Production Technology for Redclaw Crayfish (*Cherax quadricarinatus*). Final Report, Project 92/119, Fisheries Research and Development Corporation. Department of Primary Industries, Queensland, Walkamin.

LOYA-JAVELLANA GN AND FIELDER DR (1997). Developmental trends in the mouthparts during growth from juvenile to adult of the tropical freshwater crayfish, *Cherax quadricarinatus* von Martens, 1868 (Decapoda: Parastacidae). Invertebrate reproduction & development 32(2):167-175. doi: 10.1080/07924259.1997.9672619.

MEADE ME AND WATTS SA (1995). Weight gain and survival of juvenile Australian crayfish *Cherax quadricarinatus* fed formulated feeds. Journal of the World Aquaculture Society 26(4):469-474.

QUEENSLAND GOVERNMENT (2016). Ross Lobegeiger Report to Farmers. Aquaculture production summary for Queensland 2015 - 16. Page 11, Queensland Government, Brisbane.

QUEENSLAND GOVERNMENT (2018). Ross Lobegeiger report to farmers. Aquaculture production summary for Queensland 2017-18. Page 8, Queensland Government, Brisbane.

QUEENSLAND GOVERNMENT (2020a). Ross Lobegeiger report to farmers. Aquaculture production summary for Queensland 2018-19 Page 11, Queensland Government, Brisbane.

QUEENSLAND GOVERNMENT (2020b). Water Monitoring Information Portol. Department of Natural Resources, Mines and Energy
Brisbane, Queensland.

RICE PR AND ARMITAGE KB (1974). The effect of photoperiod on oxygen consumption of the crayfish *Orconectes nais* (Faxon). Comparative Biochemistry and Physiology Part A: Physiology 47(1):261-270.

RIGG DP, COURTNEY RL, SEYMOUR JE AND JONES CM (2021a). Determining Suitable Thermal Regimes for Early Instar Redclaw Juveniles, *Cherax quadricarinatus* (von Martens, 1868) (Decapoda, Parastacidae), for a Proposed Nursery Phase. Freshwater Crayfish 26(1): 1-7.

RIGG DP, COURTNEY RL, SEYMOUR JE AND JONES CM (2021b). Evaluation of Four Practical Diets on the Growth and Survival of Juvenile Redclaw, *Cherax quadricarinatus* (von Martens, 1868). Freshwater Crayfish 26(1): 1-8.

RIGG DP, COURTNEY RL, JONES CM AND SEYMOUR JE (2021c). Morphology and Weight-length Relationships for the First Six Instars of *Cherax quadricarinatus* (von Martens, 1868). Freshwater Crayfish 26(1): 1-8.

SAOUD IP, GARZA DE YTA A AND GHANAWI J (2012). A review of nutritional biology and dietary requirements of redclaw crayfish *Cherax quadricarinatus* (von Martens 1868). Aquaculture Nutrition 18(4):349-368. doi: 10.1111/j.1365-2095.2011.00925.x.

SAURABH S AND SAHOO P (2008). Major diseases and the defence mechanism in giant freshwater prawn, *Macrobrachium rosenbergii* (de Man). Proceedings of the National Academy of Sciences, India, Section B 78:103-121.

THOMPSON K, MUZINIC L, CHRISTIAN T, WEBSTER C, MANOMAITIS L AND ROUSE D (2003). Lecithin requirements of juvenile Australian red claw crayfish *Cherax quadricarinatus*. Aquaculture Nutrition 9(4):223-230.

ULLOA WALKER DA, MORALES SUAZO MC, AND EMERENCIANO MGC (2020). Biofloc technology: principles focused on potential species and the case study of Chilean river shrimp *Cryphiops caementarius*. Reviews in Aquaculture.

WHEATLEY MG AND AYERS J (1995). Scaling of calcium, inorganic contents, and organic contents to body mass during the molting cycle of the fresh-water crayfish *Procambarus clarkii* (Girard). Journal of Crustacean Biology 15(3):409-417.

8. Appendix

Chapter 5.3.2.

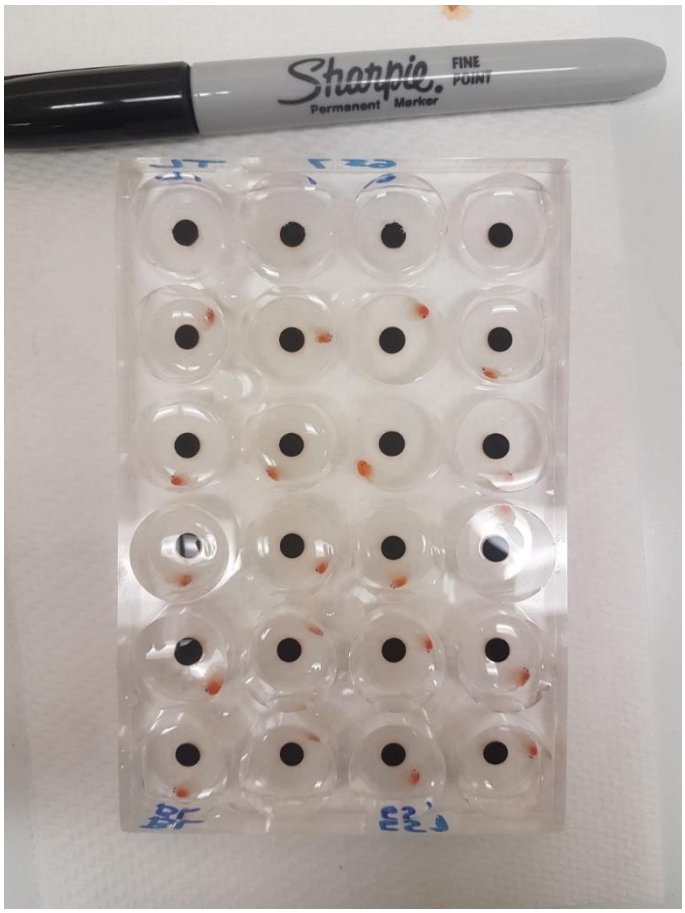

Plate 3. Microplate with 5.5 ml chambers (x 24), optodes in black in the centre of each chamber.
Microplates with 2.5 ml wells were identical except for chamber volume. The top row were run as controls with no animals. Redclaw crayfish present in the five rows below the controls are L2 stage.

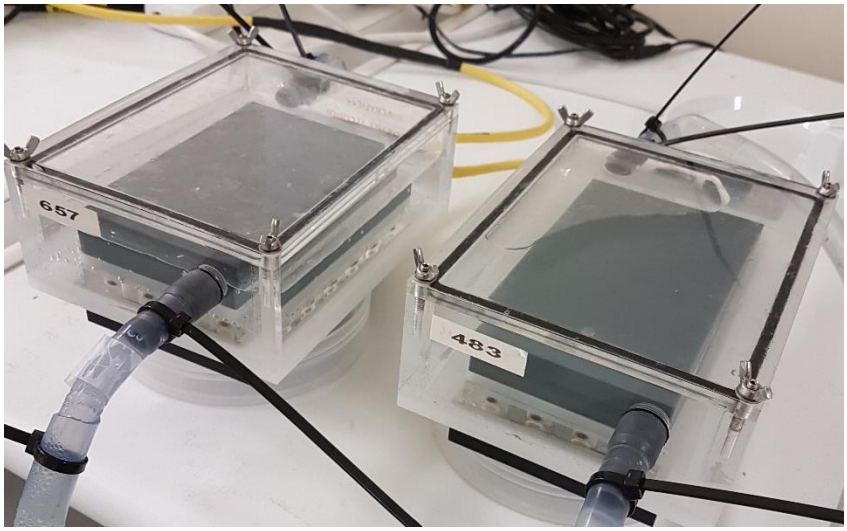

Plate 4. Metabolic rate Perspex experimental chambers.
Separate flow-through water systems with microplates in each, allowing for two separate temperature treatments to be conducted concurrently with four controls and 20 experimental animals each.

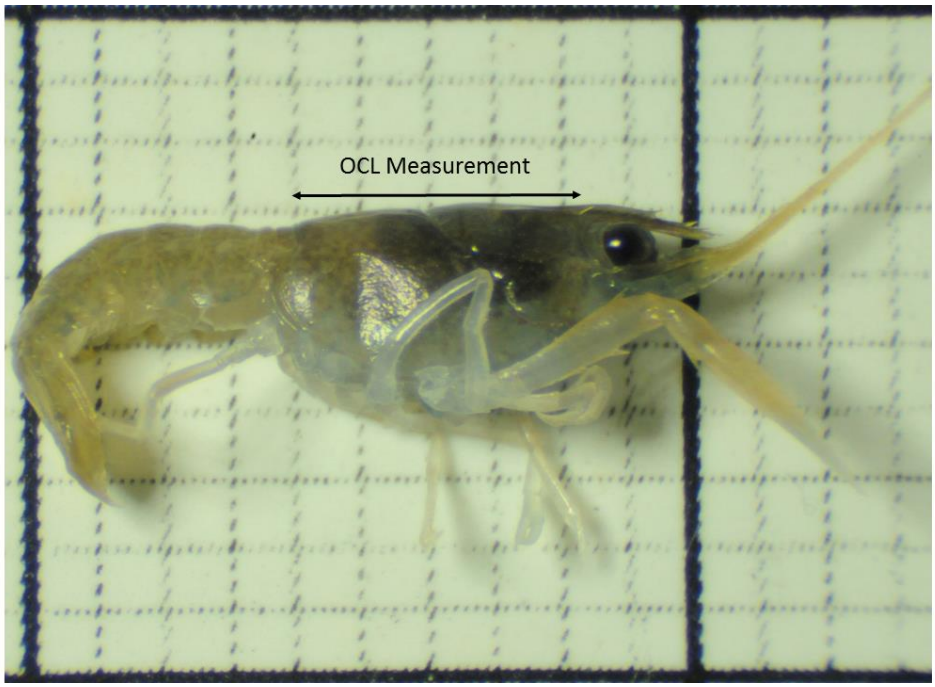

Plate 5. Ocular carapace length (OCL) measurement for *Cherax quadricarinatus*.

Chapter 5.4

Table 7. The 95% confidence interval of the regression slope between each temperature range for each instar.

Instar	Temperature Range	Slope	95% Lower C.I.	95% Upper C.I.
Egg	16°C - 20°C	-0.084	-1.39	-0.029
LI	16°C - 20°C	0.043	0.004	0.083
L2	16°C - 20°C	0.037	-0.02	0.093
J1	16°C - 20°C	0.141	0.108	0.175
J2	16°C - 20°C	0.137	0.057	0.218
J3	16°C - 20°C	-0.040	-0.095	0.015
Egg	20°C - 22°C	0.469	0.351	0.588
L1	20°C - 22°C	0.618	0.373	0.864
L2	20°C - 22°C	0.234	0.134	0.333
J1	20°C - 22°C	0.042	-0.054	0.138
J2	20°C - 22°C	-0.114	-0.302	0.075
J3	20°C - 22°C	0.336	0.255	0.417
Egg	22°C - 24°C	-0.014	-0.178	0.150
L1	22°C - 24°C	-0.139	-0.422	0.145
L2	22°C - 24°C	0.130	0.002	0.258
J1	22°C - 24°C	0.175	0.042	0.308
J2	22°C - 24°C	0.280	0.090	0.470
J3	22°C - 24°C	0.402	0.207	0.596
Egg	24°C - 26°C	-0.029	-0.222	0.163
L1	24°C - 26°C	-0.077	-0.271	0.117
L2	24°C - 26°C	0.139	-0.067	0.345
J1	24°C - 26°C	0.049	-0.090	0.188
J2	24°C - 26°C	0.169	-0.077	0.415
J3	24°C - 26°C	0.215	0.032	0.398
Egg	26°C - 28°C	-0.072	-0.254	0.111

L1	26°C - 28°C	-0.054	-0.270	0.161
L2	26°C - 28°C	-0.213	-0.446	0.020
J1	26°C - 28°C	0.395	0.250	0.540
J2	26°C - 28°C	0.057	-0.171	0.285
J3	26°C - 28°C	-0.205	-0.359	-0.051
Egg	28°C - 31°C	0.262	0.017	0.507
L1	28°C - 31°C	0.050	-0.079	0.178
L2	28°C - 31°C	0.334	0.123	0.545
J1	28°C - 31°C	0.228	0.076	0.381
J2	28°C - 31°C	0.686	0.267	1.106
J3	28°C - 31°C	0.402	0.207	0.597
Egg	31°C - 34°C	0.209	-0.035	0.454
L1	31°C - 34°C	0.283	0.047	0.519
L2	31°C - 34°C	0.223	-0.013	0.458
J1	31°C - 34°C	0.101	-0.107	0.308
J2	31°C - 34°C	0.065	-0.490	0.621
J3	31°C - 34°C	0.364	0.002	0.726
Egg	34°C - 36°C	-0.398	-0.819	0.024
L1	34°C - 36°C	0.565	0.069	1.061
L2	34°C - 36°C	1.045	0.534	1.557
J1	34°C - 36°C	0.729	0.182	1.277
J2	34°C - 36°C	0.579	-0.165	1.323
J3	34°C - 36°C	0.525	-0.290	1.340
Egg	36°C - 39°C	-0.498	-0.641	-0.356
L1	36°C - 39°C	-0.610	-0.931	-0.290
L2	36°C - 39°C	-0.547	-0.907	-0.187
J1	36°C - 39°C	-0.075	-0.368	0.217
J2	36°C - 39°C	0.175	-0.261	0.611
J3	36°C - 39°C	-0.111	-0.722	0.499
J3	39°C - 41°C	-0.969	-1.310	-0.628

Table 8. Pairwise ANCOVA comparisons performed once the band of non-significantly different intervals was established; each interval was compared to the first interval.

Instar	Temperature Range	*F* Value	*p* value
Egg	22°C- 24°C / 24°C-26°C	$F_{1, 65} = 0.016$	$p = 0.900$
Egg	24°C-26°C / 26°C-28°C	$F_{1, 57} = 0.104$	$p = 0.748$
Egg	22°C- 24°C /26°C-28°C	$F_{1, 61} = 0.231$	$p = 0.633$
L1	22°C- 24°C / 24°C-26°C	$F_{1, 71} = 0.138$	$p = 0.711$
L1	24°C-26°C / 26°C-28°C	$F_{1, 69} = 0.025$	$p = 0.874$
L1	26°C-28°C /28°C-31°C	$F_{1, 63} = 0.819$	$p = 0.369$
L1	22°C- 24°C /28°C-31°C	$F_{1, 65} = 1.524$	$p = 0.222$
L1	22°C- 24°C /26°C-28°C	$F_{1, 67} = 0.230$	$p = 0.633$
L2	20°C- 22°C /22°C- 24°C	$F_{1, 68} = 1.738$	$p = 0.192$
L2	22°C- 24°C / 24°C-26°C	$F_{1, 61} = 0.006$	$p = 0.941$
L2	20°C- 22°C /24°C-26°C	$F_{1, 66} = 0.810$	$p = 0.372$
J1	20°C- 22°C /22°C- 24°C	$F_{1, 71} = 2.827$	$p = 0.097$
J1	22°C- 24°C / 24°C-26°C	$F_{1, 68} = 1.791$	$p = 0.185$
J1	20°C- 22°C /24°C-26°C	$F_{1, 72} = 0.007$	$p = 0.932$
J2	22°C- 24°C / 24°C-26°C	$F_{1, 72} = 0.523$	$p = 0.472$
J2	24°C-26°C / 26°C-28°C	$F_{1, 75} = 0.458$	$p = 0.501$
J2	22°C- 24°C /26°C-28°C	$F_{1, 74} = 2.281$	$p = 0.135$
J3	20°C- 22°C /22°C- 24°C	$F_{1, 63} = 0.388$	$p = 0.536$
J3	22°C- 24°C / 24°C-26°C	$F_{1, 71} = 1.999$	$p = 0.162$
J3	20°C- 22°C /24°C-26°C	$F_{1, 69} = 1.257$	$p = 0.266$

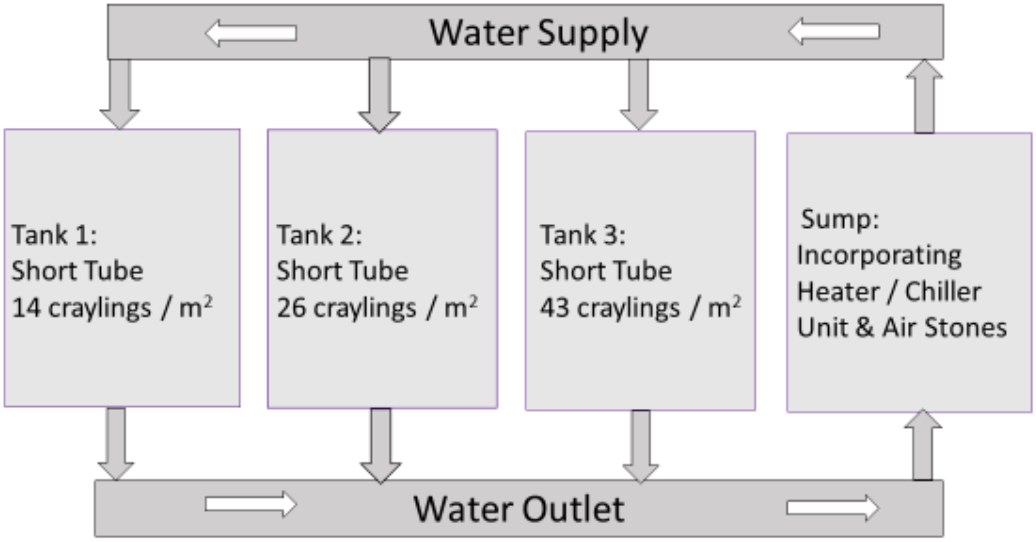

Figure 44. Habitat and density experiment 1, systems 1 and 3, tube microhabitat experiments, both systems were identical.

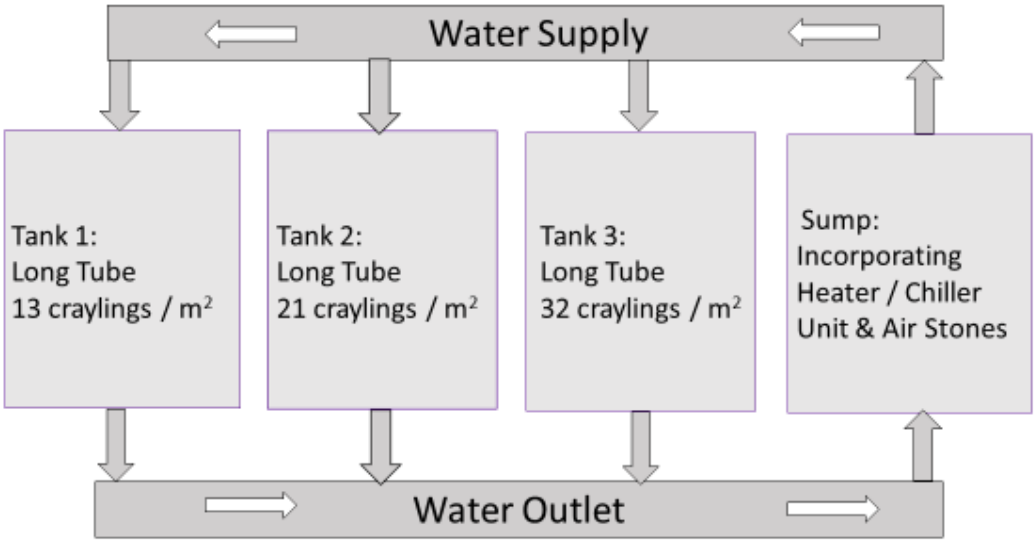

Figure 45. Habitat and density experiment 1, systems 2 and 4, tube microhabitat experiments, both systems were identical.

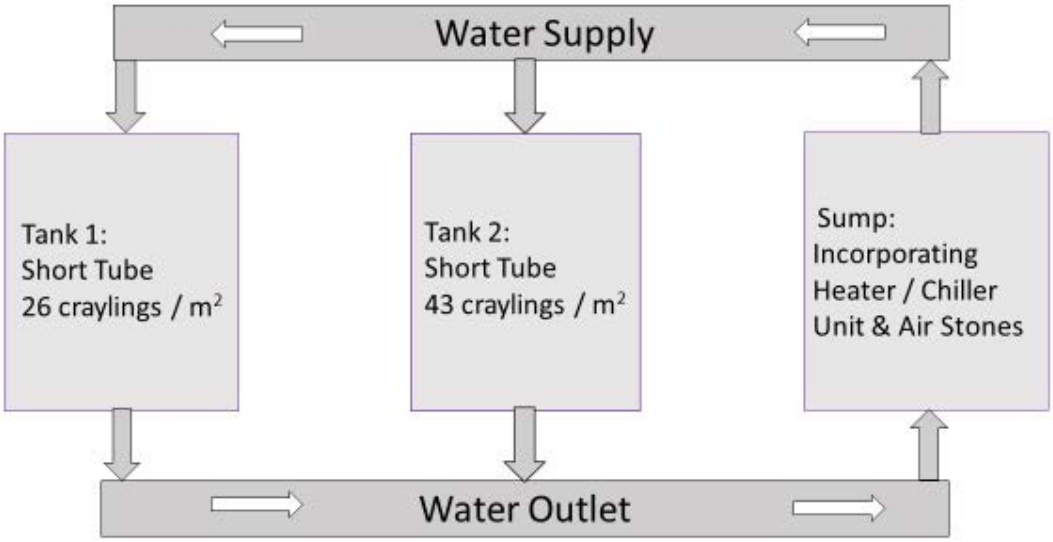

Figure 46. Habitat and density experiment 1, system 5, tube microhabitat experiment.

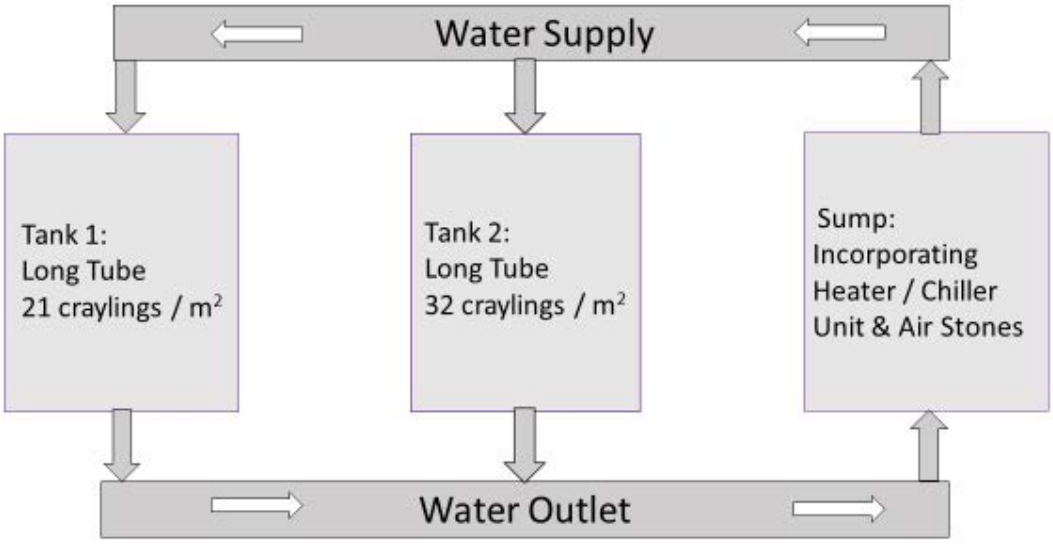

Figure 47. Habitat and density experiment 1, system 6, tube microhabitat experiment.

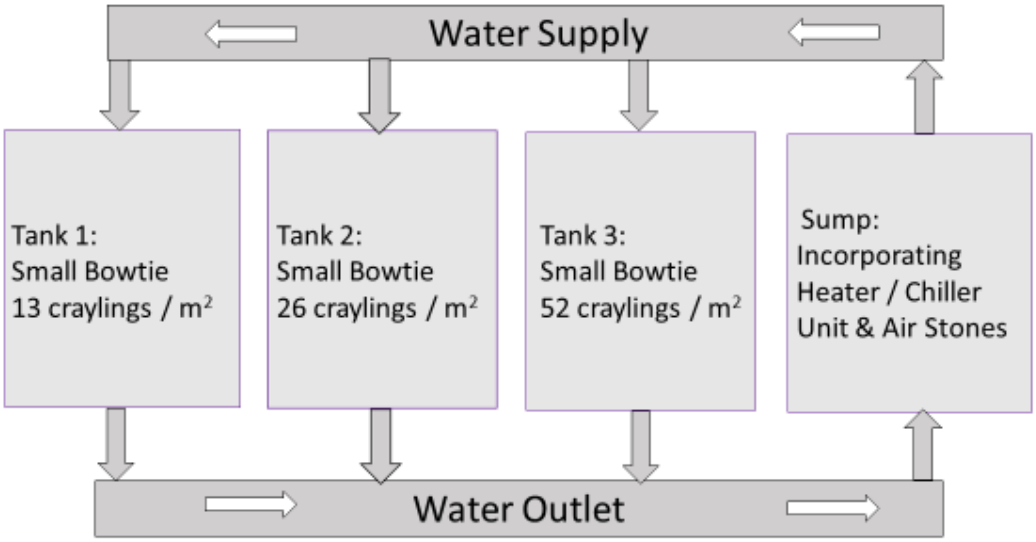

Figure 48. Habitat and density experiment 2, systems 1 – 3, bowtie microhabitat experiment, all 3 systems were identical.

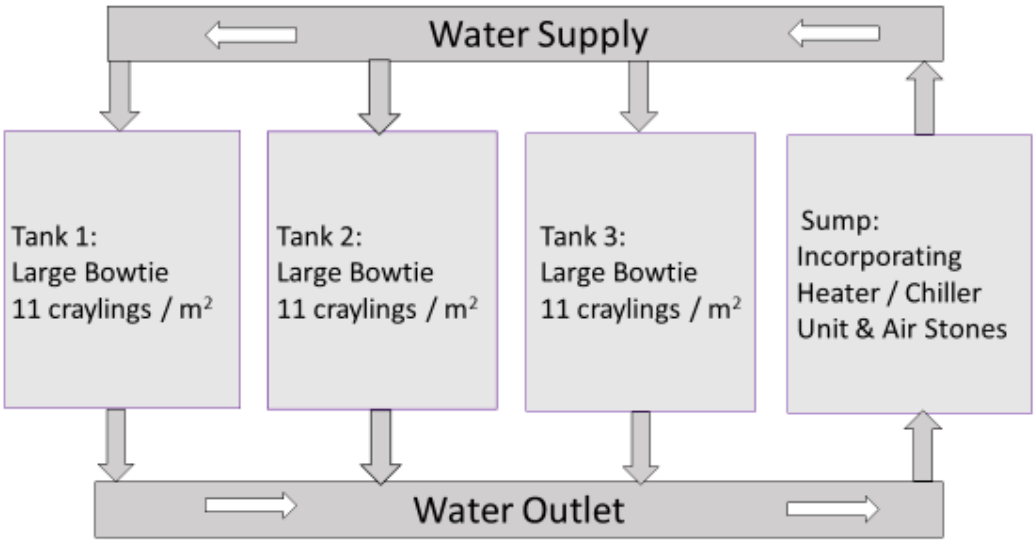

Figure 49. Habitat and density experiment 2, system 4, bowtie microhabitat experiment.

Plate 6. Habitat and density experiment 1, tube microhabitat trial, a trial habitat for nursery phase on-growing of redclaw.

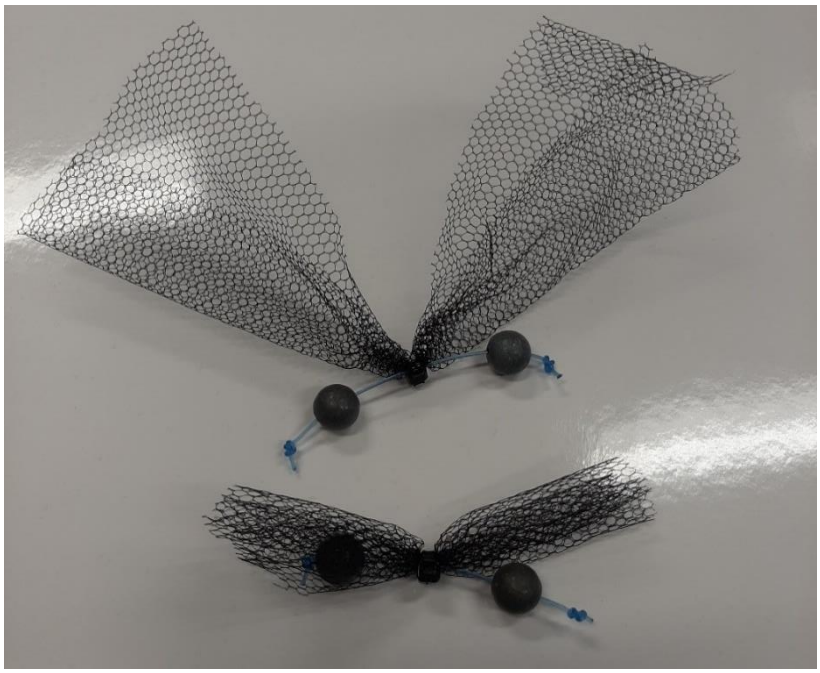

Plate 7. Habitat and density experiment 2, bowtie microhabitat, large bowtie (top of picture), small bowtie (bottom of picture) a trial habitat for nursery phase on-growing of redclaw.