Determining Suitable Thermal Regimes for Early Instar Redclaw Juveniles, *Cherax quadricarinatus* (von Martens, 1868) (Decapoda, Parastacidae), for a Proposed Nursery Phase

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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.

INTRODUCTION

Cherax quadricarinatus (von Martens, 1868)*,* 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 1990; Jones and Ruscoe 2002; Webster et al. 2004; Bugnot and López Greco 2009; Ghanawi and Saoud 2012; Saoud et al. 2013; Zhu et al. 2013; Stumpf et al. 2014). It was first trialled as an aquaculture species in the late 1980's (Jones 1990). 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 1990). The life cycle is simple, with larval development occuring 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

Table 1. Nomeclature for early life stages of redclaw applied/adopted 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 García-Guerrero et al. (2003)
L1	1	First stage after hatching	Referred to as post-embryo I by García-Guerrero et al. (2003)
L2	2	Stage after first moult	Referred to as stage 12 post-embryo II by García-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

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 growout is completed, 6 to 9 months after stocking (Jones and Valverde 2020). When survival is low, and yield therefore poor, the cause of the mortalities may be unclear. The small size and physical vulnerability of the craylings to predation is considered the most liklely. 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 growout production and yields.

As for all poikilotherms, temperature is a fundamental factor in redclaw biology and physiology, particularly in modulation of growth (Jones 1990; King 1994; Jones 1995b; Yeh and Rouse 1995; Zhao et al. 2000; García-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 (García-Guerrero et al. 2003), raising juvenile redclaw (King 1994; Jones 1995a; 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, 2013). An optimal egg incubation temperature of 22 to 25°C was recommended for redclaw by García-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.

MATERIALS AND METHODS

Juvenile terminology

For clarity, the nomeclature applied to the early life stages of redclaw in this study is defined as per Table 1.

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 × 35 mm top dimension, depth = 60 mm, 20 × 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.

Table 2. 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 (mg) ± SE Start	Mean Wt (mg) ± SE End	Mean Wt (mg) ± SE At death	Mean weight gain (%) ± SE
18°C	17.5 ± 0.1	19.8 ± 0.2	–	17.3 ± 2.0
19.5°C	17.4 ± 0.1	31.1 ± 0.5	22.3 ± 0.0	78.0 ± 3.2
22°C	17.7 ± 0.2	38.3 ± 1.2	–	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	–	19.2 ± 0.6	–

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 × 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.

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, and 32°C ± 0.5°C.

Craylings

Craylings for the experiment were hatchery-raised from eggs and supplied by AquaVerde redclaw farm in North Queensland (-17.405556, 145.525556, 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* individuals 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.

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.

$$\text{Percentage Weight Increase} = \frac{Wt_{d22} - Wt_{d1}}{Wt_{d1}} \times 100$$

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 2). Statistical analyses were conducted using IBM SPSS Statistics Version 24.

RESULTS

Water quality parameters over the course of the experiment were; Ammonia (NH_3) ≤ 0.25 ppm, Nitrite (NO_2) = 0, pH = 7.5, hardness = 0 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
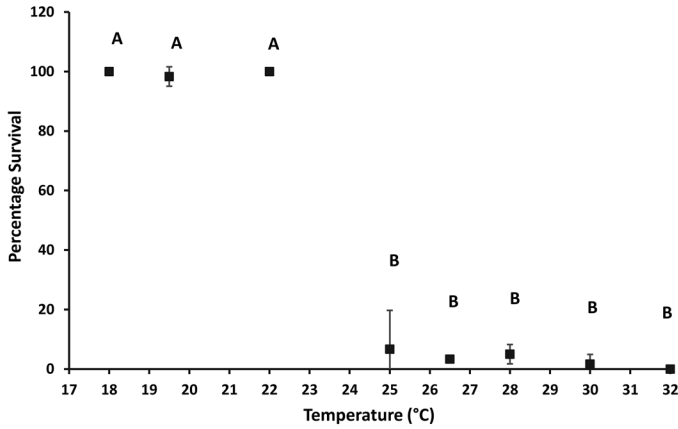
Figure 1. Percentage survival of *Cherax quadricarinatus* craylings at each of eight temperatures over the 22-day experiment. Data points are presented as means, bars represent 95% Confidence Interval, *n* = 16. Treatments with the same letter are not significantly different.
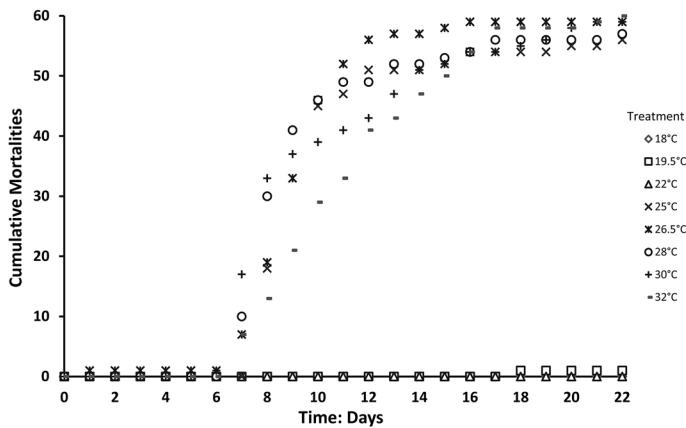

Figure 2. Cumulative crayling mortalities for *Cherax quadricarinatus* in a 22-day temperature trial. Starting number, n = 60 per temperature treatment, cumulative mortality of 60 equals 100% mortality.
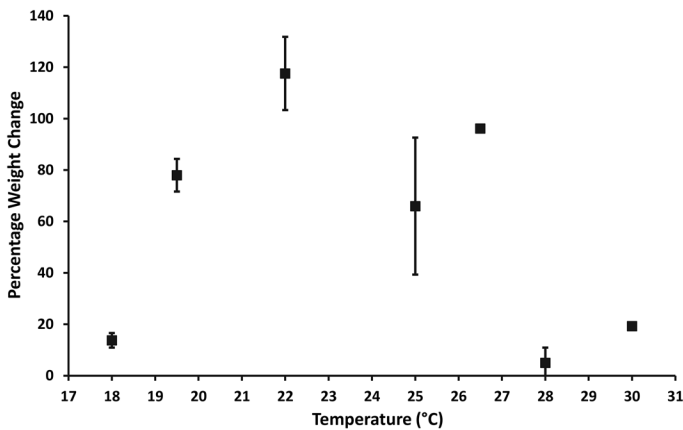

Figure 3. Weight increase of *Cherax quadricarinatus* craylings over a 22-day temperature experiment. Mean percentage gain on original weight. Bars represent 95% confidence interval (18°C, n = 60; 19.5°C, n = 59; 22°C, n = 60; 25°C n = 4; 26.5°C, n = 1; 28°C, n = 3; 30°C, n = 1; and 32°C, n = 0).

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 1).

A strong pattern emerged in the number of daily mortalities (Figure 2), 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.

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 3). 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).

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 (Figure 1). 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 2) 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 (0.75g ± 0.23g 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 1995a) showed survival highest at temperatures of 28°C and below and best growth (i.e., above 70% of maximum) at temperatures 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) and 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 1995a), 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, personal 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 (Jones 1995a). 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 larger sub-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 1995a; 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, 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 is not as counter-intuitive as it appears. Breeding activity in natural populations of redclaw begins at 21 to 22°C (Sammy 1988; Jones 1990). 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.

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