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

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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.

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INTRODUCTION

The redclaw crayfish *Cherax quadricarinatus* (von Martens, 1868) 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 1980s, redclaw were introduced into aquaculture but subsequently 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; García-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; García-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

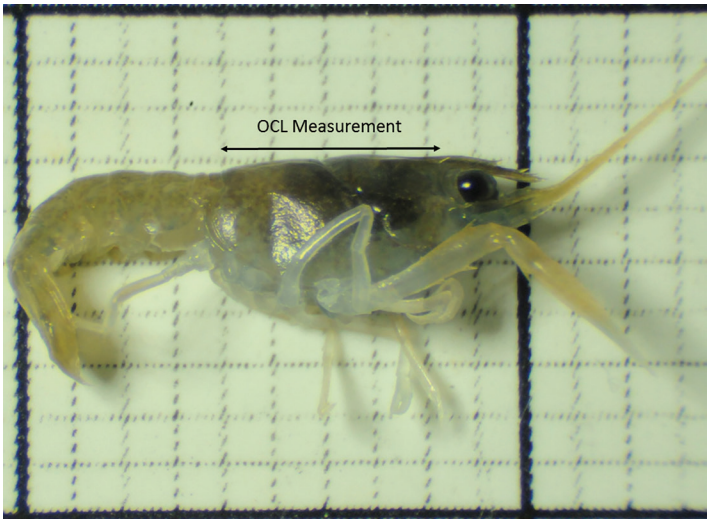

Figure 1. Ocular carapace length measurement for *Cherax quadricarinatus.*

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 morphology for identification. Nonetheless, 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, to aid in identification of slow-growing and fast-growing animals for grading and selection purposes. 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 lyophilised 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.

MATERIALS AND METHODS

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 27–28°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 27–28°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 $L \cdot h^{-1}$ 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 in individual instar cohorts, (eggs, L1, L2 and J1 were determined by morphology, J2 and J3 were determined by observation of moulting events) and 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 exogenous feeding instars (J1, J2 and J3) were fed defrosted frozen enriched *Artemia* sp. to satiation.

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 1). 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.

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 1 mm × 1 mm graticule to illustrate comparative size between instars, and the features pertaining to each instar such as differences in body morphology were also described.

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, as used by Jones and Ruscoe (1996) as the independent variable to compare against 14 morphometric measurements, and studies by Geddes et al. (1993) and Geddes et al. (1988) which established the regression relationship between OCL and wet weight. 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 lyophilised dry weight for instars egg to J3. Wet weight, OCL and lyophilised dry weight means were calculated for each instar. All statistical analyses were performed using IBM SPSS Statistics version 25.

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 (Figure 1). All instars were placed in a -80°C freezer for storage, followed by lyophilisation using a Labogene Scanvac Coolsafe for a minimum of 3 days, then weighed to determine lyophilized, dry weight.

RESULTS

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 (Figure 2A). L1 instar shows an early body form, largely undifferentiated between the cephalothorax and almost transparent abdomen, with an integral yolk sac (Figure 2B). 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 (Figure 2C). 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 (Figure 2D). Post third ecdysis, instar J2 (Figure 2E) and post fourth ecdysis instar J3 (Figure 2F) are essentially indistinguishable from instar J1, apart from an increase in size and mass.

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) = $1.01E^{-3} + 1.22E^{-4} \times$ Volume (mm^3)], ($R^2 = 0.611$, $F_{1, 189} = 296.614$, $P < 0.001$), (Figure 3). There was no significant relationship between wet weight and OCL for instar L1 [Wet Weight (g) = 1.71E-4 × OCL (mm) + $1.18E^{-2}$], ($R^2 = 0.001$, $F_{1, 167} = 0.191$, $P = 0.663$). 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) = $1.66E^{-3} \times$ OCL (mm) + $8.88E^{-3}$], ($R^2 = 0.086$, $F_{1, 176} = 13.363$, $P < 0.001$), (Figure 4). 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) = $1.86E^{-3} \times$ OCL (mm) + $1.25E^{-2}$], ($R^2 = 0.040$, $F_{1, 194} = 7.948$, $P = 0.005$), (Figure 5). There was a significant positive relationship for *C. quadricarinatus* J2 instar between wet weight and OCL [Individual Wet Weight (g) = $1.79E^{-2} \times$ OCL (mm) +-3.86E-2], ($R^2 = 0.711$, $F_{1, 190} = 467.944$, $P < 0.001$), (Figure 6). There was a significant positive relationship for *C. quadricarinatus* J3 instar between wet weight and OCL [Individual Wet Weight (g) = $2.36E^{-2} \times$ OCL (mm) + - $5.48E^{-2}$], ($R^2 = 0.681$, $F_{1, 208} = 441.690$, $P < 0.001$), (Figure 7).

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 8). Mean wet weight for each instar is significantly different and increases with increasing age $F_{5, 1640} = 1436.238$, $P < 0.001$ (Figure 9). The lyophilised dry weight of *C. quadricarinatus* did not change significantly between instars from egg to J1, however there is an increase in lyophilised 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 10). Similarly, J3 crayfish are significantly larger in terms of lyophilised dry weight than all the previous instars (Figure 10).

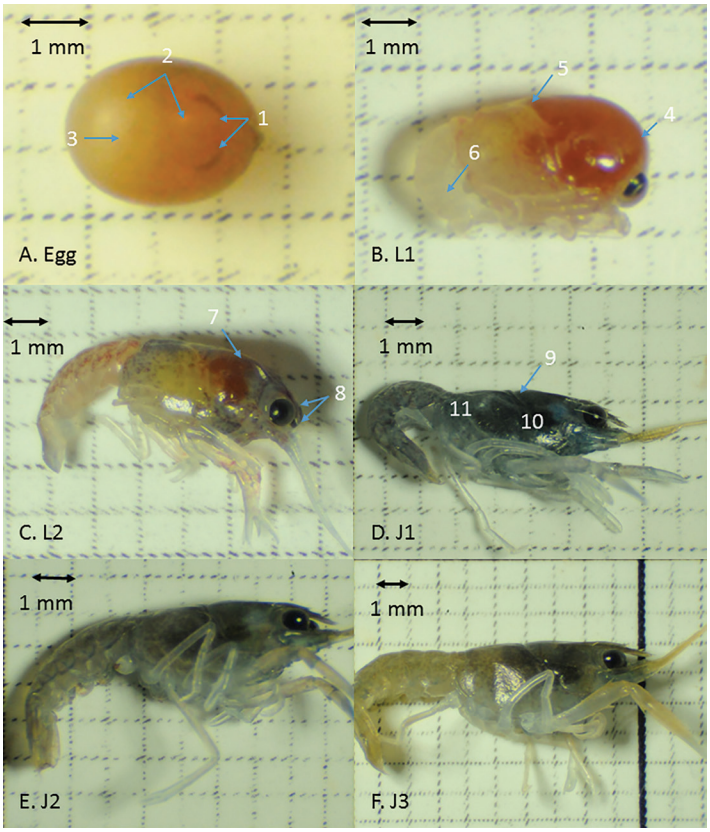

Figure 2. A, *Cherax quadricarinatus* late-stage egg showing crescent-shaped eyes (1), differentiation within the egg (2) a translucent area posterior to the eyes (3) and a general granular appearance of egg contents. **B**, *C. quadricarinatus* L1 instar, a non-mobile lecithotrophic stage, showing the 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.

Wet weight, OCL, and lyophilised dry weight means were calculated for each of the first six instars for *C. quadricarinatus* (Table 2). Wet weight increased from egg to L1, and progressively through the instars to J3 (Figure 7, Table 3). OCL also showed a progressive increase from L1 to J3, whereas lyophilised dry weight did not change until J2 instar (Figure 6, Figure 8, Table 2).

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

Table 1. 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 (García-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 (García-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 (García-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; García-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

Table 2. Mean weight and length data for the first six instars of *Cherax quadricarinatus*.

Instar		Wet Weight (g)	OCL (mm) or Egg Volume (mm^3)	Lyophilised Dry Weight (g)
Egg	*n*	192	191	163
	Mean	0.006920	48.4985	0.002874
	S.E.	0.0000651	0.41485	0.0000397
L1	*n*	174	169	163
	Mean	0.012303	2.7349	0.002915
	S.E.	0.0001004	0.02011	0.0000320
L2	*n*	253	177	230
	Mean	0.013989	2.8994	0.002926
	S.E.	0.0001138	0.02104	0.0000430
J1	*n*	197	195	185
	Mean	0.018339	3.1405	0.002871
	S.E.	0.0001635	0.01752	0.0000341
J2	*n*	197	192	194
	Mean	0.025786	3.6005	0.004248
	S.E.	0.0005242	0.02492	0.0000950
J3	*n*	215	209	198
	Mean	0.043601	4.1789	0.007507
	S.E.	0.0007679	0.02700	0.0001756

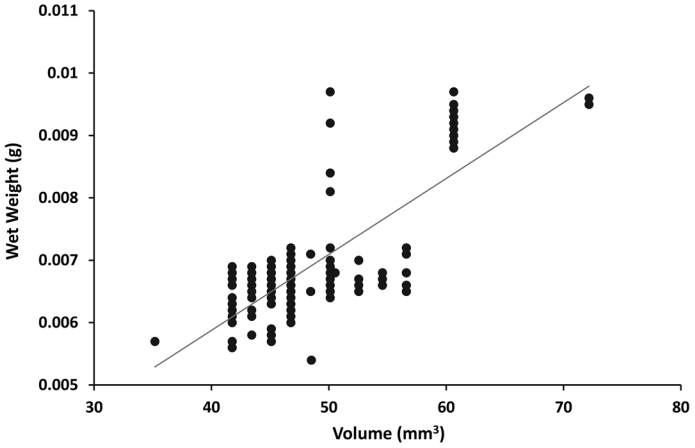

Figure 3. Bivariate relationship for *Cherax quadricarinatus* eggs, the predictor is individual egg volume, the response variable is individual wet weight.

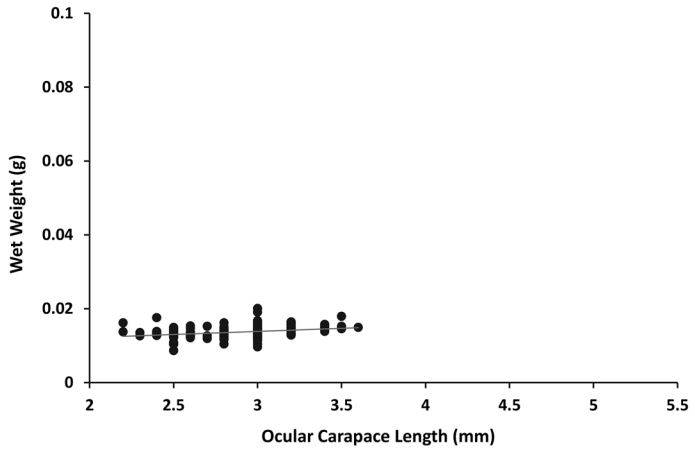

Figure 4. Bivariate relationship for *Cherax quadricarinatus* L2 instar, the predictor is individual ocular carapace length, the response variable is individual wet weight.

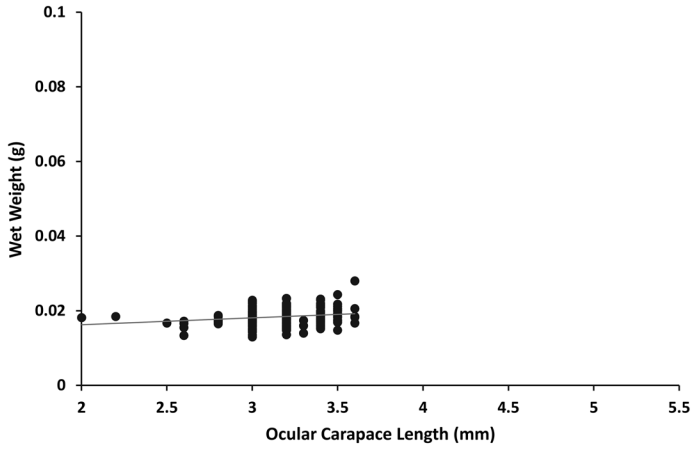

Figure 5. Bivariate relationship for *Cherax quadricarinatus* J1 instar, the predictor is ocular carapace length, the response variable is individual wet weight.

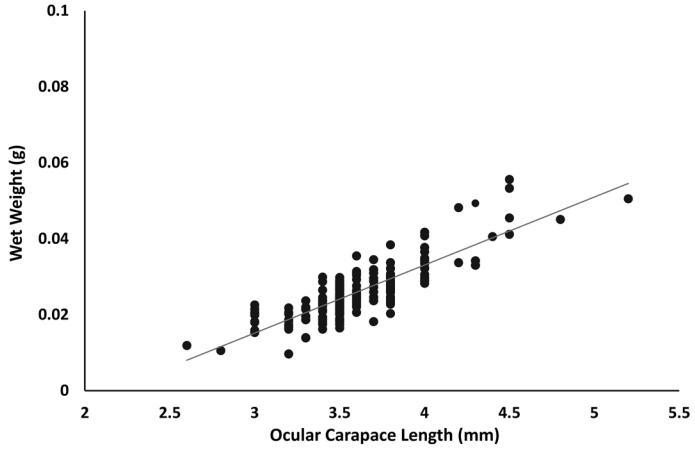

Figure 6. Bivariate relationship for *Cherax quadricarinatus* J2 instar, the predictor is individual ocular carapace length, the response variable is individual wet weight.

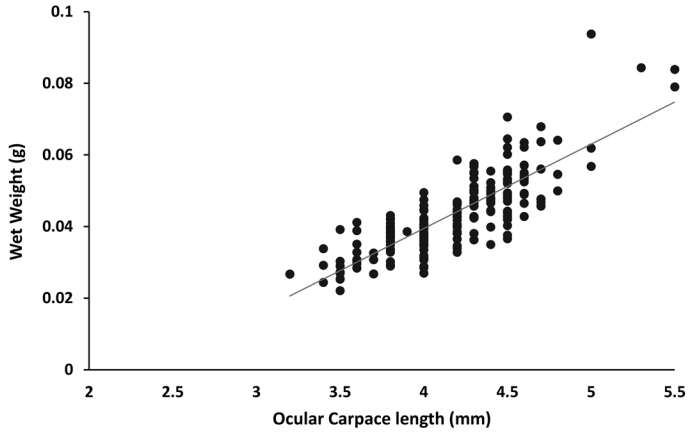

Figure 7. Bivariate relationship for *Cherax quadricarinatus* J3 instar, the predictor is individual ocular carapace length, the response variable is individual wet weight.

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.

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; García-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) (Figure 1). 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, as any given stage may vary according to genetic stock and environmental and nutrtional factors.

For five of the six instars examined, there was a significant relationship between wet weight and OCL which allows for

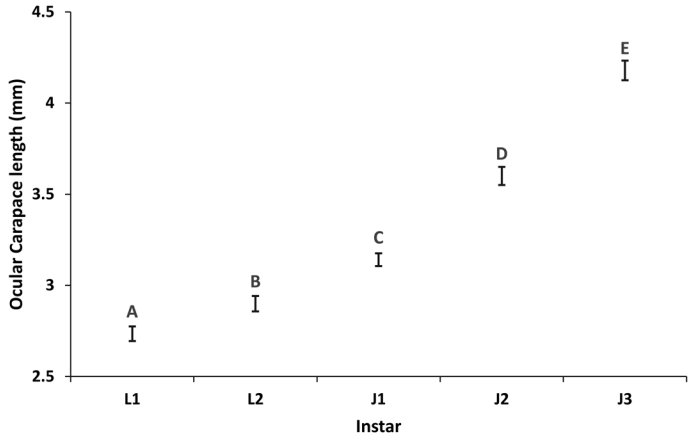

Figure 8. OCL means for the first five hatched instars of redclaw *Cherax quadricarinatus*, error bars represent ± 2 S.E., bars with the same letter are not significantly different.

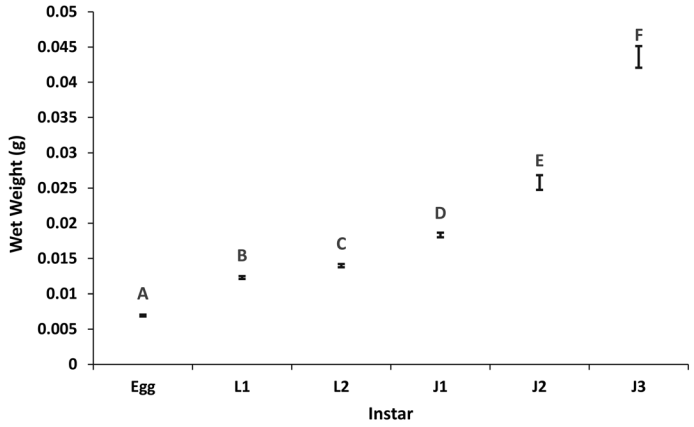

Figure 9. Wet weight means for the first six instars of redclaw *Cherax quadricarinatus*, error bars represent ± 2 S.E., bars with the same letter are not significantly different.

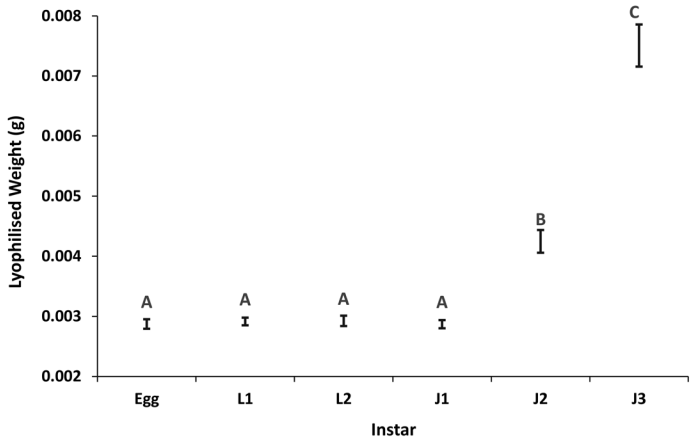

Figure 10. Lyophilised dry weight means for the first six instars of redclaw *Cherax quadricarinatus*, error bars represent ± 2 S.E., bars with the same letter are not significantly different.

computation (with varying accuracy) 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 of *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 in identifying 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 seperated 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, realtive 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 2.

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 (García-Guerrero et al. 2003), OCL increases significantly with each successive instar from egg (Figure 6), wet weight increases significantly with each successive instar from egg (Figure 7), and lyophilised dry weight remains unchanged between egg and J1, increasing significantly at J2 and at J3 (Figure 8). With no apparent nutrtional input from the external environment for instars egg, L1 and L2, 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 ruptering 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.

CONCLUSION 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, that 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, that 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.

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