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ORIGINAL ARTICLE

Haemolymph constituents and osmolality as functions of moult stage, body weight, and feeding status in marron, *Cherax cainii* (Austin and Ryan, 2002) and yabbies, *Cherax destructor* (Clark, 1936)

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KEYWORDS

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Abstract The study investigates the change in osmolality and haemolymph constituents in marron *Cherax cainii* and yabbies *Cherax destructor* associated with moult stages, body weights and their feeding status. A total of 582 haemolymph samples from 5 moult stages (postmoult-AB, intermoult-C, and premoult stages – D_0, D_1, D_2), two body weight classes (2–15 g and 61–75 g) and nutritional status were used for analysis of osmolality, protein, glucose, and ionic concentrations of potassium and chloride following the standard biochemical procedures. The haemolymph protein, glucose, potassium and chloride levels were highest at intermoult and early premoult stages, and lowest at postmoult in both crayfish species. Except protein, no significant differences were seen in analyzed parameters between various weight classes and two species. Haemolymph osmolality, protein and glucose were significantly higher in fed crayfish, whereas no variations in haemolymph potassium and chloride concentrations were observed between the fed and unfed crayfish. Maximum osmolality was recorded at 7–8 h after feeding in both crayfish species. The results showed that the biochemical changes in the haemolymph of marron and yabbies are related to moult stages, body weight and feeding and thus can be used as tools for determining suitable diets.
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1. Introduction

Marron *Cherax cainii* (Austin and Ryan, 2002) and yabbies *Cherax destructor* (Clark, 1936) are two crayfish species, indigenous to Australian fresh water habitats (Bryant and Papas, 2007) and are important species for aquaculture, especially in Western Australia (Johnson, 1986; Mills, 1980). The growth and development of crayfish are functions of a number of intrinsic factors including moult stages, body weights and

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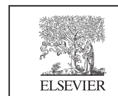
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feeding status. Change in haemolymph constituents during the life cycle and culture environment are used as health status indicators in a number of crustaceans (Charmantier-Daures and Vernet, 2004; Charmantier et al., 1994; Lignot et al., 1999, 2000). The haemolymph parameters such as osmolality, protein, glucose, sodium, potassium and chloride are commonly monitored parameters in several crustacean species and their values have been determined to correlate with moult cycle, body weight and nutritional status (Lignot et al., 1999; Marcy et al., 2009; Pascual et al., 2006; Pratoomchat, 2002; Skinner, 1985; Vargas-Albores and Ochoa, 1982).

The regulation of haemolymph protein, glucose, potassium and chloride are also important physiological mechanisms that can easily be affected by variations of culture conditions. Lignot et al. (1999) concluded that haemolymph glucose from the shrimp *Penaeus stylirostris* was dependant on the species nutritional status, while protein concentration from haemolymph of the giant river prawn *Macrobrachium rosenbergii* was a function of weight, and moult stage (Cheng et al., 2002). Changes in haemolymph electrolyte levels have been examined in the American lobster *Homarus americanus*, the giant river prawn *M. rosenbergii* and blue crab *Callinectes sapidus* during the moult cycle (Mercaldo-Allen, 1991; Sugumar et al., 2013; Towle and Mangum, 1985). Increases in haemolymph osmolality of Cl^-, Na^+, K^+, Ca^{2+} and Mg^{2+} have been observed as functions of size and moult cycle in mud crab *Scylla serrata* (Chen and Chia, 1997).

Starvation can affect crustacean haemolymph constituents due to nutrient deficiency (Lignot et al., 1999; Sugumar et al., 2013). The induced starvation of crustaceans in the intermoult stage has been suggested to be a good way to understand any biochemical and physiological adaptations during the starvation mode (Barclay et al., 1983).

However, no studies are available to determine the effects of moult stage, body weight and feeding status on the concentration of protein, glucose, potassium and chloride in the haemolymph of marron and yabbies. The biochemical changes occurring in crustaceans during moulting, feeding and starvation are indicators of their nutritional requirements and are an important basis for determining suitable diets (Sugumar et al., 2013). Further, it is imperative to understand the variation in haemolymph physiology and biochemistry with moult stages, body weights, and nutritional status in order to manage them efficiently in any aquaculture situation.

The aim of this research was to describe the changes in osmolality, protein, glucose, K^+ and Cl^- concentrations in the haemolymph of marron and yabbies associated with moult stages, body weights and the feeding status.

2. Materials and methods

A total of 300 marron and 300 yabbies used in the experiments were procured from farms in Colie (200 km from Perth city with longitude 33°22′43.40″ S and latitude 116°15′07.49″ E), Western Australia and transported to Curtin Aquatic Research Laboratory (CARL) for acclimation prior to experiments. They were stocked in 800 L round water tanks (diameter 120 cm × height 70 cm) in the presence of PVC pipes of appropriate sizes (10–15 cm length and 3–8 cm diameter) to provide shelter and avoid cannibalism during moulting.

During acclimation, the water quality was kept at an optimum level by controlling temperature (24 ± 2 °C), saturated oxygen (8.3 ± 0.7 mg L^{-1}) and photoperiod of 12 h of light and dark cycle. The amount of water loss due to siphoning and evaporation was topped up regularly. The crayfish were fed *ad libitum* with commercial formulated pellets (24.5% of crude protein-CP, 6% lipid and 7.5% ash, 21.2 kJ g^{-1} gross energy, 11.5 mg CP kJ^{-1} protein/energy ratio and 8.5% moisture) on alternate days.

Examination of moult stages

Prior to the moult stage examination, individual crayfish were placed in crushed ice to render them inactive. Each animal was then placed on its back in a petri dish, the uropods flattened, covered with distilled water and a cover slip, and setae on the apical quarter of the uropod margin were then examined under a compound microscope (Leica Microsystem DM 2500-German at x100 magnification). Five discrete moult stages: postmoult (AB), intermoult (C), premoult (D_0, D_1, D_2) were identified by examining setal development and changes in epidermal retraction state on the uropods according to Drach (1939), Drach and Tchernigovtzeff (1967) and further modified by Ha et al. (unpublished). The main characteristics used to define the moult stages were AB: setal base is clearly visible, thin cuticle, epidermal tissue is visible inside the setal lumen; C: thick cuticle layer at setal bases where the epidermis lies just underneath; D_0: a translucent space to form between the old cuticle and the epidermis; epidermis retraction from cuticle started; D_1: retraction of epidermis from is maximal, new setae appear and are clearly visible; D_2: the appearance of the new, folded cuticle layer and the new setae are visible.

Haemolymph sampling and analyzes

Approximately one hundred (100) μL of haemolymph was withdrawn from the pericardial cavity of crayfish through the intersegment membrane between the cephalothorax and the first abdominal segment using 0.5 mL syringe. Haemolymph osmolality was measured by injecting a 20 μl sample into a micro-osmometer (Model 3MO plus, Advance Instruments, Norwood, MA, USA) and expressed in mosm kg^{-1}. Haemolymph protein was determined using the Bio-Rad Protein Assay Kit No. 500-0006 Bio-Rad Laboratories, Richmond, CA, USA using bovine albumin (molecular weight: 66,000) as a standard, a method derived from Bradford (1976). Glucose concentration from haemolymph was analyzed using reflectance photometer Glucometer 3 from Bayer Diagnostics, AMES Department and Glucofilm sticks. Haemolymph potassium was determined using Horiba K^+ meter. Chloride was quantified using Photometer LF 2400.

Crayfish were divided into 3 experiments to cover the aim of the current study:

Experiment 1: Moult stage and body weight on haemolymph constituents

Two weight classes for each species: 2–15 g (marron: 9.8 ± 2.4 g, n = 75; yabbies: 9.4 ± 1.7 g, n = 65) and 60–75 g (marron: 67.8 ± 3.1 g, n = 55; yabbies: 65.9 ± 3.5 g, n = 65) were selected for the experiment. After a 24 h starvation period, crayfish were collected from the experimental tanks in batches of 10 for haemolymph sampling. Each haemolymph sample was noted relative to moult stage (AB, C, D_0, D_1,

D_2) and wet weight. Between 14 and 39 haemolymph samples were collected for each of the moult stages (Table 1). Osmolality and haemolymph constituents: protein, glucose, potassium and chloride were analyzed after sampling.

Experiment 2: The effect of feeding and starvation on osmolality, protein, glucose, K^+, Cl^-

Marron and yabbies at intermoult stage C were divided into the fed (marron: 63.7 ± 2.6 g, $n = 20$; yabbies: 61.9 ± 3.4, $n = 20$) and starved (marron: 67.5 ± 3.9, $n = 20$; yabbies: 63.5 ± 4.9 g, $n = 20$) groups. Animals from both fed and staved groups were under 24 h starvation prior to experimentation. The concentration of osmolality, protein, glucose, K^+, Cl^- was investigated 4 h after feeding *ab-libitum*.

Experiment 3: The effect of feeding and starvation on osmolality over 550 min after feeding

Only marron and yabbies at intermoult stage (C) were used for the experiment. The crayfish were divided into the fed and starved group (marron: 63.7 ± 2.6 g, $n = 130$; yabbies: 61.8 ± 3.4 g, $n = 130$). Batches of 10 marron and 10 yabbies were collected at regular intervals (every 30 min) from fed and starved groups (24 h of starvation prior to experimentation) for sampling haemolymph. Time (minute) of each haemolymph sampling for osmolality measurement was recorded.

Statistical analyzes

SPSS 18.0, 2014 was used to analyze the data. Results were presented as mean ± SE. The normality of data was assessed by the Shapiro–Wilk test (Winer, 1991) and the homogeneity of variance was assessed by Levene test (Winer, 1991) prior to the analyzes. One-way ANOVA (analyzes of variance) and LSD (least significant difference) post hoc tests were used to determine significant differences among sized groups and moult stages. To satisfy the assumptions of normality and/or homogeneity of variance data were transformed to $\log_{10}(x + 1)$. All significant tests were at $P < 0.05$ level.

3. Results

Experiment 1: Moult stage and body weight on osmolality and haemolymph constituents

Protein concentration from crayfish haemolymph was the highest at intermoult (C) (Table 1) and had a minimum concentration at postmoult (AB). The difference in weights of crayfish did not have any impact on the level of haemolymph protein. A significant difference in haemolymph protein between marron and yabbies in all moult stages and from each weight class occurred.

Glucose concentration in haemolymph varied significantly ($P < 0.05$) throughout moult cycle (Table 2). The highest levels of glucose were in intermoult (C) and early premoult (D_0) stages and significantly lower in the late premoult (D_2) and postmoult (AB) stages in both marron and yabbies. Glucose level was independent of weight classes and species.

Haemolymph K^+ and Cl^- were significantly higher during intermoult stage (C) (Table 3). No significant difference in K^+ was recorded in both crayfish species and weight classes. Cl^- also increased significantly from postmoult (AB) to intermoult (C), and then decreased significantly ($P < 0.05$) by nearly 10% at stage D_2. Both crayfish species at different body weight classes showed similar changes in chloride concentration over the entire moult cycle.

Experiment 2: The effect of feeding and starvation on haemolymph osmolality, glucose, K^+, and Cl^-

The haemolymph osmolality, protein and glucose of both fed crayfish showed a significant increase after feeding (Table 4). No significant difference in haemolymph potassium and chloride concentrations was observed between fed and starved crayfish.

Experiment 3: Variation of osmolality during 550 min after feeding

The osmolality of starved marron and yabbies stayed constant during the 550 min of feeding experiment (marron: $y = 0.032\ x + 463.8$, $P > 0.05$, $R^2 = 0.11$; and yabbies: $y = 0.025\ x + 411.5$, $P > 0.05$, $R^2 = 0.06$) (Figs. 1 and 2). In contrast, the osmolality of fed marron and yabbies significantly increased in the first 485 min after feeding (marron: $y = 0.017\ x + 484.1$, $P < 0.05$, $R^2 = 0.76$; and yabbies: $y = 0.24\ x + 426.1$, $P < 0.05$, $R^2 = 0.81$) and then significantly decreased to 540 min (marron: $y = -0.52x + 696.2$, $P < 0.05$, $R^2 = 0.68$; and yabbies: $y = -0.47x + 643.3$, $P < 0.05$, $R^2 = 0.80$). The osmolality of the fed crayfish was within the range of variation of the values of the starved groups. The maximum change of haemolymph osmolality was registered 480 min after feeding.

4. Discussion

The stable and favorable laboratory conditions (controlled water temperature of 24 °C and dissolved oxygen of 8.3 mg L^{-1}) used in this experiment should have no bearing

Table 1 Haemolymph protein concentration (mg mL^{-1}) in different weight classes and moult stages of marron and yabbies.

Weight (g)	Species	AB	C	D_0	D_1	D_2
2–15	Marron	$_1115.5 \pm 3.6^a$ (17)	$_1148.6 \pm 3.7^b$ (19)	$_1143.5 \pm 2.7^b$ (13)	$_1134.7 \pm 3.2^b$ (12)	$_1117.5 \pm 3.6^a$ (11)
	Yabbies	$_2132.7 \pm 2.3^a$ (18)	$_2158.2 \pm 2.5^b$ (21)	$_2157.2 \pm 3.1^b$ (15)	$_1139.4 \pm 3.6^a$ (14)	$_2134.6 \pm 4.1^a$ (13)
61–75	Marron	$_1110.2 \pm 3.6^a$ (18)	$_1144.9 \pm 2.8^b$ (22)	$_1136.4 \pm 4.2^b$ (10)	$_1128.4 \pm 3.8^b$ (16)	$_1113.5 \pm 4.2^a$ (14)
	Yabbies	$_2125.3 \pm 3.9^a$ (13)	$_2156.6 \pm 2.2^b$ (18)	$_2152.3 \pm 2.9^b$ (12)	$_1132.1 \pm 3.8^a$ (17)	$_2129.1 \pm 3.8^a$ (13)

Same alphabetical superscripts (a, b) in the same row (comparisons among moult stages) and numerical subscripts (1, 2) in the same column (comparisons among weight classes and species) are not significantly different at the $P = 0.05$ level. Numbers in the brackets are animals used for the measurements.

Table 2 Haemolymph glucose concentration (mg mL^{-1}) in different weight classes and moult stages of marron and yabbies.

Weight (g)	Species	AB	C	D_0	D_1	D_2
2–15	Marron	$_1 1.2 \pm 0.3^a$	$_1 3.2 \pm 0.2^b$	$_1 2.9 \pm 0.2^b$	$_1 1.7 \pm 0.2^a$	$_1 1.4 \pm 0.2^a$
	Yabbies	$_1 1.0 \pm 0.2^a$	$_1 3.8 \pm 0.1^b$	$_1 3.5 \pm 0.6^b$	$_1 1.8 \pm 0.3^a$	$_1 1.2 \pm 0.3^a$
61–75	Marron	$_1 0.7 \pm 0.2^a$	$_1 3.7 \pm 0.9^b$	$_1 3.4 \pm 0.7^b$	$_1 1.9 \pm 0.1^a$	$_1 0.9 \pm 0.2^a$
	Yabbies	$_1 0.8 \pm 0.3^a$	$_1 4.0 \pm 0.4^b$	$_1 2.7 \pm 0.1^b$	$_1 2.1 \pm 0.2^a$	$_1 1.1 \pm 0.3^a$

Same alphabetical superscripts (a, b) in the same row (comparisons among moult stages) and numerical subscripts in the same column (comparisons among weight classes and species) are not significantly different at the $P = 0.05$ level. Numbers of measurements (not mentioned) are same as in Table 1.

Table 3 Haemolymph K^+ and Cl^- concentration in different weight classes and moult stages of marron and yabbies.

Factor	Weight (g)	Species	AB	C	D_0	D_1	D_2
Potassium (mmol L^{-1})	2–15	Marron	$_1 3.0 \pm 0.8^a$	$_1 4.9 \pm 0.3^b$	$_1 3.8 \pm 0.4^a$	$_1 3.0 \pm 0.4^a$	$_1 3.1 \pm 0.3^a$
		Yabbies	$_1 3.1 \pm 0.4^a$	$_1 4.3 \pm 0.2^b$	$_1 3.5 \pm 0.2^a$	$_1 3.4 \pm 0.8^a$	$_1 3.3 \pm 0.5^a$
	61–75	Marron	$_1 2.8 \pm 0.3^a$	$_1 4.6 \pm 0.3^b$	$_1 2.9 \pm 0.2^a$	$_1 2.9 \pm 0.6^a$	$_1 3.2 \pm 0.3^a$
		Yabbies	$_1 3.2 \pm 0.6^a$	$_1 5.1 \pm 0.4^b$	$_1 3.4 \pm 0.6^a$	$_1 3.7 \pm 0.4^a$	$_1 3.5 \pm 0.4^a$
Chloride (mmol L^{-1})	2–15	Marron	$_1 169.2 \pm 5.2^a$	$_1 190.9 \pm 4.1^b$	$_1 180.2 \pm 3.3^a$	$_1 174.5 \pm 4.7^a$	$_1 172.6 \pm 4.9^a$
		Yabbies	$_1 174.6 \pm 3.5^a$	$_1 190.8 \pm 3.2^b$	$_1 182.5 \pm 2.7^a$	$_1 175.5 \pm 5.6^a$	$_1 176.8 \pm 4.3^a$
	61–75	Marron	$_1 165.4 \pm 4.2^a$	$_1 185.9 \pm 2.5^b$	$_1 172.4 \pm 3.6^a$	$_1 170.6 \pm 4.2^a$	$_1 168.7 \pm 5.5^a$
		Yabbies	$_1 175.6 \pm 5.3^a$	$_1 196.4 \pm 2.9^b$	$_1 182.4 \pm 2.8^a$	$_1 175.8 \pm 4.1^a$	$_1 175.6 \pm 4.5^a$

Same alphabetical superscripts (a, b) in the same row (comparisons among moult stages) and numerical subscripts in the same column of each parameters (comparisons among weight classes and species) are not significantly different at the $P = 0.05$ level. Numbers of measurements (not mentioned) are same as in Table 1.

Table 4 Haemolymph osmolality, protein, glucose, potassium and chloride concentrations for starved and fed (4 h after feeding) marron and yabbies. n = number of measurements.

Species	Nutritional condition	Weight	Osmolality (mosm kg^{-1})	Protein (mg mL^{-1})	Glucose (mg L^{-1})	Potassium (mg L^{-1})	Chloride (mg L^{-1})
Marron	Starved (n = 20)	67.5 ± 3.9	516 ± 12^a	127.4 ± 4.6^a	47 ± 7.5^a	3.7 ± 0.6^a	186.7 ± 6.5^a
	Fed (n = 20)	63.7 ± 2.6	582 ± 10^b	109.1 ± 2.9^b	65 ± 18^b	3.9 ± 0.5^a	181.9 ± 4.7^a
Yabbies	Starved (n = 20)	63.5 ± 4.9	501 ± 10^a	131.9 ± 3.7^a	45 ± 12^a	3.4 ± 1.7^a	191.8 ± 7.5^a
	Fed (n = 20)	61.9 ± 3.4	557 ± 11^b	113.7 ± 6.1^b	58 ± 14^b	3.2 ± 1.1^a	193.6 ± 5.5^a

Same alphabetical superscripts (a, b) in the same column of each species are not significantly different at the $P = 0.05$ level. n = number of measurement.

on the changes in haemolymph osmolality. Therefore, the changes in osmolality during the experiment are solely due to the moult stages and other applied experimental variables (Charmantier et al., 1994; Lignot et al., 1999).

Crustaceans have to face the influence of the moult cycle on their internal environment during their entire life cycle (Aiken and Waddy, 1992; Bliss, 1985; Franco et al., 2006; Garcia, 1988; Passano, 1960). The effects of moult cycle on haemolymph protein levels have been observed in European green crab *Carcinus maenas* (Busselen, 1970), pink shrimp *Penaeus duorarum* (Bursey and Lane, 1971), American lobster *H. americanus* (Mercaldo-Allen, 1991), mud crab (*S. serrate*) (Chen and Chia, 1997), white leg shrimp *Litopenaeus vannamei* (Cheng et al., 2002; Galindo et al., 2009), and in giant river prawn *M. rosenbergii* (Cheng et al., 2001). Similarly, the haemolymph protein level is also affected by the nutritional status in European lobster *H. gammarus* (Hagerman, 1983), *C. maenas* (Busselen, 1970) and brown shrimp *Crangon vulgaris* (Djangmah, 1970), and in blue swimmer crab *Portunus pelagicus* (Sugumar et al., 2013).

In the present study, haemolymph protein levels of marron and yabbies were the lowest at postmoult stage (AB), and the highest at intermoult stage (C) and early premoult (D_0). These results were similar to results from *M. rosenbergii* (Cheng et al., 2001) and *L. vannamei* (Cheng et al., 2002). However, our results were different to the protein levels observed in *P. japonicus* and *P. duorarum* which were highest at early postmoult stage (D_0), and lowest at postmoult stage (AB) (Bursey and Lane, 1971; Chen and Cheng, 1993). Shortly before ecdysis or late premoult (D_2), a peak value for haemolymph protein of *C. vulgaris* is reached (Djangmah, 1970). A threefold dilution of haemolymph protein in *H. vulgaris* at the time of ecdysis was observed by Glynn (1968). A reduction in haemolymph protein from 80–90 to 30 mg mL^{-1} in *C. vulgaris* during ecdysis was observed by Djangmah (1970). Nicol (1967) estimated that at ecdysis of a 100 g *Carcinus maenas* with a premoult haemolymph volume of 37 mL, 70–80 mL of water is taken in, resulting in two to three times dilution of the haemolymph protein. A decrease in haemolymph protein

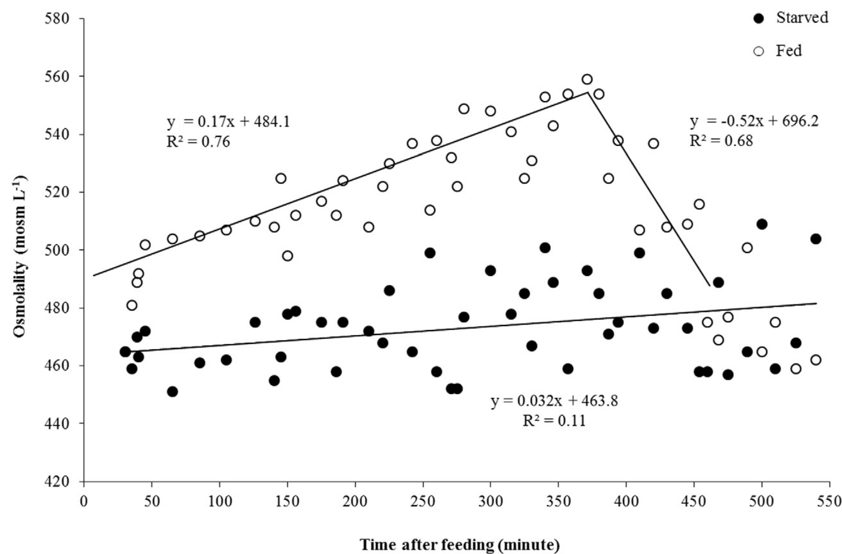

Figure 1 Change in osmolality of fed and starved marron with time (minutes) after feeding. The lines represent the linear regressions best fitting the data.

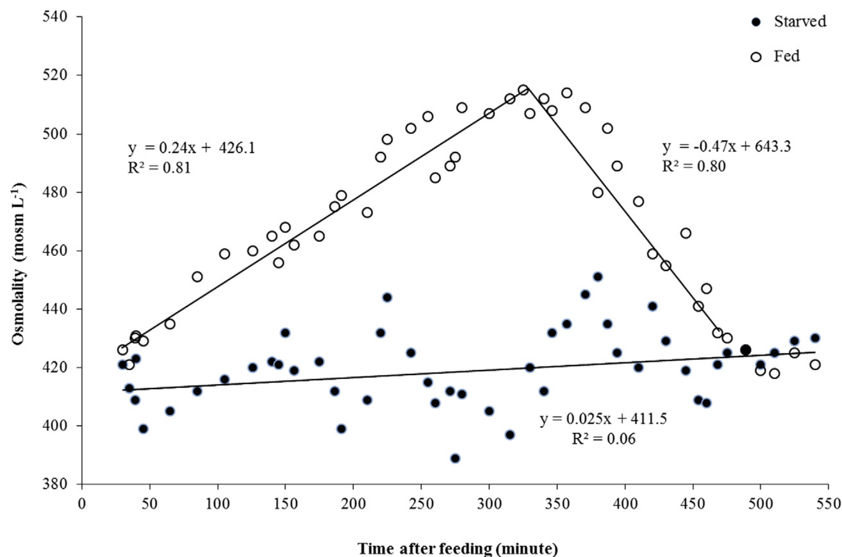

Figure 2 Change in osmolality of fed and starved yabbies according to time (minutes) after feeding. The lines represent the linear regressions best fitting the data.

level in marron and yabbies during postmoult stage (AB) is also due to water uptake following the action of Na^+/K^+-ATPase which can establish an osmotic gradient by the influx of water across epithelia (Towle and Mangum, 1985).

Glucose is a molecule that has a major role in the energy metabolism of crustaceans (Galindo et al., 2009) and its variations in the haemolymph are related to the quantity and quality of carbohydrates contained in the diet (Rosas et al., 2000). We found a great variability in glucose concentrations (0.7–4.0 mg mL^{-1}), and significant differences in glucose during the moult cycles of both crayfish. In contrast, the giant tiger prawn *Penaeus monodon* (Ferraris et al., 1987) and *L. vannamei* (Cheng et al., 2002) maintained isosmotic conditions, showing no significant differences of glucose concentrations throughout their moult cycles. In the present study, the level of glucose in the haemolymph increased during intermoult (C) and early premoult stage (D_0), while a decline was observed during postmoult (AB), which is similar in *H. americanus* (Telford, 1968) and *P. pelagicus* (Sugumar et al., 2013). The postmoult decrease in glucose may be due to its utilisation as a precursor in chitin synthesis (Hornung and Stevenson, 1971; Meenakshi and Scheer, 1961). In the purple shore crab *Hemigrapsus nudus* the major portion of the required chitin is synthesized in the postmoult period by incorporating glucose carbon into chitin during the early postmoult period (Meenakshi and Scheer, 1961), while the synthesis of chintin from glucose reached a peak at postmoult stage in the allegheny crayfish *Orconectes obscurus* (Hornung and Stevenson, 1971).

The variability of the haemolymphatic ionic composition throughout the moult cycle in different crustaceans has been

known for a long time (Baumberger and Olmsted, 1928). During the premoult (D_0, D_1 and D_2) in most of the crustaceans, the haemolymph ionic composition is affected by reabsorption and partial excretion of mineral components from the calcified cuticle (Greenaway, 1993; Wheatly, 1999). Most of electrolytic elements tend to be lower in postmoult (AB), due to an uptake of water at ecdysis (Bliss et al., 1966; Cameron, 1989; Charmantier-Daures and Vernet, 2004; Chen and Chia, 1997; Cheng et al., 2001, 2002; Mantel and Farmer, 1983; Neufeld and Cameron, 1992). The present study also showed that K^+, Cl^- levels are significantly higher at the postmoult stage (AB) and early premoult (D_0). During the intermoult stage (C), there is a decrease in integument permeability creating an impermeable barrier between the internal and outside environment, however at the same time there is an increase in ATPase activity which induces mobilisation of ionic secretion into the haemolymph (Charmantier et al., 1994). Moreover, during late premoult (D_2) and early postmoult stages an increase in permeability of the integument (Hunter and Uglow, 1993) allows water absorption (Ferraris et al., 1987), that results in a dilution of ion concentration in haemolymph and causes a reduction in the osmotic capacity leading to an internal environment closer to the isosmotic stage (Galindo et al., 2009). This explanation is confirmed by Charmantier et al. (1994) suggesting that in late premoult and early postmoult stages, changes in integument permeability and activity of Na^+/K^+-ATPase pump induced by crustaceans hyperglycaemic hormone (Lucu and Towle, 2003) takes place in the posterior gills allowing water and ions to enter tissues, resulting in an increase in body water volume after moulting. Thus, the water content of haemolymph decreases during the intermoult C (Sardà and Cros, 1984) resulting in a higher concentration of haemolymph electrolytes.

In order to expand a new and soft exoskeleton, aquatic decapods must take up water immediately during postmoult (AB). It is therefore expected that the activity of a whole range of physiological processes related to water and ion permeability and regulation vary during the moult cycle. A number of ions have been measured in the haemolymph during the cycle and most appear to be lower in concentration during the postmoult than in premoult (Chen and Chia, 1997; Cheng et al., 2001, 2002; Engel, 1987; Ferraris et al., 1987; Glynn, 1968; Mercaldo-Allen, 1991).

Feeding appeared to be another important variable controlling the osmotic regulation of the crayfish. Under the laboratory conditions, feeding of marron and yabbies was followed by an increase in haemolymph osmolality, protein, and glucose, which is similar to the fact that the haemolymph protein level decreases during starvation (Adiyodi, 1969; Djangmah, 1970; Stewart et al., 1967; Uglow, 1969). However, no variation in the concentration of either K^+ or Cl^- was observed in fed and unfed crayfish. Therefore, part of the increase in haemolymph osmolality originates from the increased protein and glucose level and not due to ionic exchange between the haemolymph and the external medium as no changes in K^+ and Cl^- concentrations occurred after feeding in the haemolymph but are linked with rapid and massive transport of organic molecules including digestive products such as glucose from the gut to the haemolymph. Similar results have been observed by Ahearn and Maginniss (1977) in *M. rosenbergii*, Lignot et al. (1999) in *P. stylirostris* and Rosas et al. (1995) in the white shrimp *Penaeus setiferus*. The osmolality increase in a few hours after feeding is therefore related to storage within the hepatopancreas and/or to direct intake by organs such as muscles (Lignot et al., 1999).

In the current study, the effects of feeding on the haemolymph osmolality appeared to be time-dependent as there was an elevation of crayfish haemolymph osmolality after 8 h of feeding. Whereas, an increase in haemolymph osmolality of *M. rosenbergii* (Ahearn and Maginniss, 1977) and *P. stylirostris* (Lignot et al., 1999) took place 4 h after feeding. Several studies have investigated the time required by the crustacean to return to conditions similar to those maintained before a meal (Mantel and Farmer, 1983). The rate at which food clears in the digestive track after a single meal has been described in some penaeids as a reason to return to pre-fed status. In *Palaemonetes varians*, this rate varied according to the food composition but not by the food particle size. Total clearance of food from the digestive track occurs 4–6 h after a detritus or algal meal and 27 h after an animal protein pelleted shrimp meal (Snow and Williams, 1971). Our study showed that the time required for both marron and yabbies to compensate for the haemolymph osmotic alteration due to the pelleted feed is approximately 7–8 h.

5. Conclusions

Haemolymph protein, glucose and electrolyte levels differ with the moult stages of marron and yabbies with no influence by their body weight classes. Lower levels of haemolymph protein, glucose, K^+, Cl^- during the postmoult period are associated with the water uptake at moulting. Starvation results in a reduced level of haemolymph protein, glucose and osmolality, with no effects on ionic constituents. Time required for both marron and yabbies to compensate for the haemolymph osmotic alteration due to the pelleted feed is approximately 7–8 h.

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References

Adiyodi, R.T., 1969. On the storage and mobilization of organic resources in the hepatopancreas of a crab *Paratelphusa hydrodromus*. Experientia 25, 43–44.

Ahearn, G.A., Maginniss, L.A., 1977. Kinetics of glucose transport by the perfused mid-gut of the freshwater prawn *Macrobrachium rosenbergii*. J. Physiol. 271, 319–336.

Aiken, D.E., Waddy, S.L., 1992. The growth process in crayfish. Rev. Aquat. Sci. 6, 335–381.

Austin, C.M., Ryan, S.G., 2002. Allozyme evidence for a new species of freshwater crayfish of the genus *Cherax Erichson* (Decapoda: Parastacidae) from the south–west of Western Australia. Invertebr. Syst. 16, 357–367.

Barclay, M.C., Dall, W., Smith, D.M., 1983. Changes in lipid and protein during starvation and the Molting cycle in the tiger

prawn, *Penaeus esculentus* Haswell. J. Exp. Mar. Biol. Ecol. 68, 229–244.
Baumberger, J.P., Olmsted, J.M.D., 1928. Changes in the osmotic pressure and water content of crabs during the molt cycle. Physiol. Zool. 1, 531–544.
Bliss, D.E., 1985. Integument, pigments, and hormonal processes. In: Bliss, D.E., Mantel, L.H. (Eds.), . In: The Biology of Crustacea, vol. 9. Academic Press, New York, pp. 3–549.
Bliss, D.E., Wang, S.M., Martinez, E., 1966. Water balance in the land crab, *Gecarcinus lateralis*, during the intermolt cycle. Am. Zool. 6 (2), 197–212.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Bryant, D., Papas, P., 2007. Marron *Cherax cainii* (Austin) in Victoria – a literature review, Arthur Rylah Institute for Environmental Research Technical Report Series No. 167. Department of Sustainability and Environment, Heidelberg.
Bursey, C.R., Lane, C.E., 1971. Ionic and protein concentration changes during the molt cycle of *Penaeus duoragrum*. Comp. Biochem. Physiol. A 40, 155–162.
Busselen, P., 1970. Effects of moulting cycles and nutritional conditions on haemolymph proteins in *Carcinus maenas*. Comp. Biochem. Physiol. 37, 73–83.
Cameron, J.N., 1989. Post-moult calcification in the blue crab, *Callinectes sapidus*: timing and mechanism. J. Exp. Biol. 143, 285–304.
Charmantier, G., Soyez, C., Aquacop, 1994. Effect of moult stage and hypoxia on osmoregulatory capacity in the peneid shrimp *Penaeus vannamei*. J. Exp. Mar. Biol. Ecol. 178, 233–246.
Charmantier-Daures, M., Vernet, G., 2004. Moulting, autotomy, and regeneration. In: Forest, J., Vaupel Klein, J.C. (Eds.), The Crustacea. Traité De Zoologie, vol. 7. Brill, Leiden, pp. 161–255.
Chen, J.C., Cheng, S.Y., 1993. Studies on haemocyanin and haemolymph protein levels of *Penaeus japonicus* based on sex, size and moulting cycle. Comp. Biochem. Physiol. 106B, 293–296.
Chen, J.C., Chia, P.G., 1997. Oxyhemocyanin, protein, osmolality and electrolyte levels in the hemolymph of *Scylla serrata* in relation to size and molt cycle. J. Exp. Mar. Biol. Ecol. 217, 93–105.
Cheng, W., Liu, C.H., Cheng, C.H., Chen, J.C., 2001. Haemolymph oxyhaemocyanin, protein, osmolality and electrolyte levels of *Macrobrachium rosenbergii* in relation to size and moult stage. Aquaculture 198, 387–400.
Cheng, W., Liu, C.H., Yan, D.F., Chen, J.C., 2002. Haemolymph oxyhaemocyanin, protein, osmolality, and electrolyte levels of white leg shrimp *Litopenaeus vannamei* in relation to size and moult stage. Aquaculture 211 (1–4), 325–339.
Clark, E., 1936. The freshwater and land crayfishes of Australia. Memoirs of the Natural History Museum, Victoria 10, 5–58.
Djangmah, J.S., 1970. The effects of feeding and starvation on copper in the blood and hepatopancreas, and on blood proteins of *Crangon vulgaris* (Fabricius). Comp. Biochem. Physiol. 32, 709–731.
Drach, P., 1939. Mue et cycle d'intermue chez les crustacés décapodes. Ann. Inst. Oceanogr. 19, 103–388 (in French with English abstract).
Drach, P., Tchernigovtzeff, C., 1967. Sur la méthode de détermination des stades d'intermue et son application générale aux Crustacés. Vie Milieu 18, 595–610 (in French with English abstract).
Engel, D.W., 1987. Metal regulation and molting in the blue crab, *Cullinectes sapidus*: copper, zinc, and metallothionein. Biol. Bull. 172, 69–82, Woods Hole, Massachusetts.
Ferraris, R.P., Parado-Estepa, F.D., de Jesus, E.G., Ladja, J.M., 1987. Osmotic and chloride regulation in the haemolymph of the tiger prawn *Penaeus monodon* during moulting in various salinities. Mar. Biol. 95, 377–385.
Franco, A.R., Ferreira, J.G., Nobre, A.M., 2006. Development of a growth model for penaeid shrimp. Aquaculture 259, 268–277.
Galindo, C., Gaxiola, G., Cuzon, G., Xavier, C.C., 2009. Physiological and biochemical variations during the moult cycle in juvenile *Litopenaeus vannamei* under laboratory conditions. J. Crust. Biol. 29, 544–549.
Garcia, S., 1988. Tropical penaeid prawns. In: Gulland, J.A. (Ed.), Fish Population Dynamics: The Implications for Management. Wiley-Interscience Publications, pp. 219–246.
Glynn, J.P., 1968. Studies on the ionic, protein and phosphate changes associated with the moult cycle of *Homarus vulgaris*. Comp. Biochem. Physiol. 26, 937–946.
Greenaway, P., 1993. Calcium and magnesium balance during moulting in land crabs. J. Crust. Biol. 13, 191–197.
Hagerman, L., 1983. Haemocyanin concentration of juvenile lobsters (*Homarus gammarus*) in relation to moulting cycle and feeding conditions. Mar. Biol. 77, 11–17.
Hornung, D., Stevenson, J., 1971. Changes in the rate of chitin synthesis during the crayfish molting cycle. Comp. Biochem. Physiol. 40B, 341–346.
Hunter, D.A., Uglow, R.F., 1993. A technique for the measurement of total ammonia in small volume of seawater and haemolymph. Ophelia 37, 31–40.
Johnson, H.T., 1986. Australian freshwater crustaceans with potential for culture – some aspects of their biology, with particular reference to the yabby. In: Proc. 1st Aust. Freshwater Aquaculture Workshop, Narrandera, 1983. Dept of Agriculture, New South Wales.
Lignot, J.H., Cochard, J.C., Soyez, C., Lemaire, P., Charmantier, G., 1999. Osmoregulatory capacity according to nutritional status, molt stage and body weight in *Penaeus stylirostris*. Aquaculture 170, 79–92.
Lignot, J.H., Spanings-Pierrot, C., Charmantier, G., 2000. Osmoregulatory capacity as a tool in monitoring the physiological condition and the effect of stress in crustaceans. Aquaculture 191, 209–245.
Lucu, C., Towle, D.W., 2003. $Na^+ + K^+$-ATPase in gills of aquatic crustacea. Comp. Biochem. Physiol. 135A, 195–214.
Mantel, L.H., Farmer, L.L., 1983. Osmotic and ionic regulation. In: Farmer, L.L. (Ed.), Internal Anatomy and Physiological Regulation. Academic Press, pp. 53–161.
Marcy, N.W., Huong, D.T.T., Jasmani, S., Jayasankar, V., Kaneko, T., Aida, K., Hatta, T., Nemoto, S., Wigginton, A., 2009. Haemolymph osmolality, ion concentrations and calcium in the structural organisation of the cuticle of the giant freshwater prawn *Macrobrachium rosenbergii*: Changes with the molt cycle. Aquaculture 292, 104–110.
Meenakshi, V., Scheer, B., 1961. Metabolism of glucose in the crabs *Cancer magister* and *Hemigrapsus nudus*. Comp. Biochem. Physiol. 3, 30–41.
Mercaldo-Allen, R., 1991. Changes in the blood chemistry of the American lobster, *Homarus americanus*, H. Milne Edwards, 1837, over the molt cycle. J. Shellfish Res. 10, 147–156.
Mills, B., 1980. Notes on the aquaculture of yabbies. In: Proc. 1st Aust. Freshwater Aquaculture Workshop, Narrandera, 1983. Dept of Agriculture, New South Wales.
Neufeld, D.S., Cameron, J.N., 1992. Postmoult uptake of calcium by the blue crab (*Callinectes sapidus*) in water of low salinity. J. Exp. Biol. 171, 283–299.
Nicol, J.A., 1967. The Biology of Marine Animals. Pitman, London, 699 pp.
Pascual, C., Sánchez, A., Zenteno, E., Cuzon, G., Gabriela, G., Brito, R., Gelabert, R., Hidalgo, E., Rosas, C., 2006. Biochemical, physiological, and immunological changes during starvation in juveniles of *Litopenaeus vannamei*. Aquaculture 251, 416–429.
Passano, L.M., 1960. Metabolism and growth. In: Waterman, T.H. (Ed.), The Physiology of Crustacea, vol. I. Academic Press, New York, pp. 473–536, 670 pp.
Pratoomchat, B., Sawangwong, P., Pakkong, P., Machado, J., 2002. Organic and inorganic compound variations in haemolymph, epidermal tissue and cuticle over the molt cycle in *Scylla serrata* (Decapoda). Comp. Biochem. Physiol. A 131, 243–255.

Rosas, C., Bolongaro-Crevenna, A., Sanchez, A., Gaxiola, G., Soto, L., Escobar, E., 1995. Role of digestive gland in the energetic metabolism of *Penaeus setiferus*. Biol. Bull. 189, 168–174.

Rosas, C., Cuzon, G., Gaxiola, G., Arena, L., Lemaire, P., Soyez, C., Van Wormhoudt, A., 2000. Influence of dietary carbohydrate on the metabolism of juvenile *Litopenaeus stylirostris*. J. Exp. Mar. Biol. Ecol. 249, 181–198.

Sardà, F., Cros, M.L., 1984. Calcium and magnesium metabolism during the moult in *Nephrops norvegicus* (L.). Inv. Pesq. 48 (3), 377–397.

Skinner, D.M., 1985. Moulting and regeneration. In: Bliss, D.E., Mantel, L.H. (Eds.), . In: The Biology of Crustacea, vol. 9. Academic Press, New York, pp. 44–146.

Snow, N.B., Williams, J., 1971. A simple method to determine the O:N ratio of small marine animals. Mar. Biol. Assoc. U. K. 51, 105–109.

Stewart, J.E., Cornick, J.W., Foley, D.M., Li, M.F., Bishop, C.M., 1967. Muscle weight relationship to serum proteins, hemocytes and hepatopancreas in the lobster, *Homarus americanus*. J. Fish. Res. Board Can. 24, 2339–2354.

Sugumar, V., Vijayalakshmi, G., Saranya, K., 2013. Molt cycle related changes and effect of short term starvation on the biochemical constituents of the blue swimmer crab *Portunus pelagicus*. Saudi J. Biol. Sci. 20, 93–103.

Telford, M., 1968. Changes in blood sugar composition during the Molt cycle of the lobster, *Homarus americanus*. Comp. Biochem. Physiol. 26, 917–926.

Towle, D.W., Mangum, C.P., 1985. Ionic regulation and transport ATPase activities during the molt cycle in the blue crab *Callinectes sapidus*. J. Comp. Physiol. 5, 216–223.

Uglow, R.F., 1969. Haemolymph protein concentrations in portunid crabs – II. The effects of imposed fasting on *Carcinus maenas*. Comp. Biochern. Physiol. 31, 959–967.

Vargas-Albores, F., Ochoa, J.L., 1982. Variation of pH, osmolality, sodium and potassium concentrations in the haemolymph of sub-adult blue shrimp *Penaeus stylirostris* according to size. Comp. Biochem. Physiol. A 102, 1–5.

Wheatly, M.G., 1999. Calcium homeostasis in Crustacea: the evolving role of branchial, renal, digestive and hypodermal epithelia. J. Exp. Biol. 283, 620–640.

Winer, B.J., 1991. Statistical Principles in Experimental Design, 3rd ed. McGraw-Hill, New York.