Int Aquat Res (2021) 13:195–207
https://doi.org/10.22034/IAR.2021.1926075.1146

ORIGINAL RESEARCH

Effects of handling stress and moulting cycle on reproductive performance and digestive capacity of female giant freshwater prawn, *Macrobrachium rosenbergii*

Noor Azlina Kamaruding . Mhd Ikhwanuddin Abdullah

Received: 14 March 2021 / Accepted: 06 August 2021 / Published online: 15 August 2021

Abstract Energy metabolism is a critical component in assessing the fundamental aspect of organism fitness in prawn aquaculture farming. This is particularly important to maintain sustainable energy use to cope with stress and tolerance without affecting growth and reproduction functions. This study aims to determine and compare energy utilisation with a special focus on reproductive performance and digestive capacity concerning the handling-stress and moulting cycle of female Giant Freshwater Prawn, *Macrobrachium rosenbergii*. There were two experimental groups, which are (i) grow-out pond-reared (G1): prawns were sampled randomly and concurrently for one day at an outdoor condition, and (ii) short-term maintenance (G2): prawns were transported to an indoor hatchery, acclimated for two weeks, and sampled by staggered-method for two consecutive months. For the respective experimental conditions, haemolymph from five females was sampled at eight different moulting stages, whereby each stage was classified as reproductive and non-reproductive moulting cycles. Cultivated females at the grow-out pond had higher levels of haemolymph glucose ($P < 0.05$) at Sub-stages D1 (0.51 ± 0.12 mg/mL), D3 (0.50 ± 0.05 mg/mL), and A (0.71 ± 0.09 mg/mL) during the reproductive moulting cycle compared to the short-term maintenance at similar moulting stages. Other findings showed that cultivated prawns at the grow-out pond had significantly ($P < 0.05$) higher glucose levels at Sub-stages D3 (0.57 ± 0.09 mg/mL) and B (0.37 ± 0.03 mg/mL) during the non-reproductive moulting cycle compared to the short-term maintenance indicating that energy was utilised to cope with the handling stress. We suggest that short-term maintenance may reduce the stressors factor and is applicable for hatchery operations to maintain the health of the prawn.

Keywords Acclimation . Glucose . Haemolymph . Macrobrachium rosenbergii . Moulting . Stress

Introduction

In recent years, giant freshwater prawn, *Macrobrachium rosenbergii* hatcheries in Malaysia met high demand for seeds. Based on the statistics provided by the Food and Agricultural Organization (FAO 2009), the global farming production capacity of *M. rosenbergii* in Asia has increased more than 99% in 2007. China (124 520 tons), Thailand (27 650 tons), India (27 262 tons), and Bangladesh (23 240 tons) were the primary producers. Meanwhile, Malaysia is one of the three *M. rosenbergii*-producing countries with 246 tons of production, Iran with 258 tons of production, and Brazil with 230 tons of production. However, insufficient and unreliable *M. rosenbergii* seed supply remained the emerging issue in Malaysia (Banu and

Noor Azlina Kamaruding (✉)
Institute of Marine Biotechnology, Universiti Malaysia Terengganu , 21030 Kuala Nerus, Terengganu, Malaysia
e-mail: noor.azlina@umt.edu.my

Mhd Ikhwanuddin Abdullah(✉)
Institute of Tropical Aquaculture, Universiti Malaysia Terengganu , 21030 Kuala Nerus, Terengganu, Malaysia
e-mail: anilza_scientist@yahoo.com

Christianus 2016). The dependency of commercial hatcheries on either adult prawns from grow-out ponds or wild broodstock from rivers increases since spawning activity will lead to a shortage of natural spawners. The issue is critical as most of the hatcheries in Malaysia prefer wild broodstock compared to the largest and healthiest ovigerous females selected from farmed broodstock (Banu and Christianus 2016). This is because seed production at the highest level was achieved by channelling low energy for gonad maturity. Most of the local hatcheries relied on wild spawners resulting in a scarcity of wild broodstock, and the efforts to revive the population are limited.

Energy metabolism plays a pivotal role in the survival of aquatic invertebrates in response to stress and tolerance (Sokolova 2013). The fitness of organisms is determined by the equal use of the conversion rate of storage and metabolic transformation of energy in an efficient manner (Troha and Ayres 2020). Therefore, it is crucial to understand the regulation of energy expenditure and its allocation to different functions such as the moulting cycle for a fundamental aspect of fitness knowledge. In Palaemonidae, including *M. rosenbergii*, the moulting cycle can be divided into two modes, which are reproductive and non-reproductive. The reproductive moulting cycle occurs when the shedding of the exoskeleton is synchronously accompanied by ovarian development. Whereas merely shed exoskeleton for growth without ovary development is called non-reproductive moulting cycle (Kamaruding et al. 2017; Kamaruding et al. 2018a)

Glucose is routinely used in aquaculture to monitor the changes in the metabolic state associated with the moulting cycle, reproductive performance, index of stress in response to a variety of stressors such as trawling, repeated handling, emersion, fishing, commercial maintenance, and temperature during transport (Mercier et al. 2006; Lorenzen et al. 2007; Lund et al. 2009, Lorenzon et al. 2013, Kamaruding et al. 2017; Cook et al. 2019). This information is crucial in providing specific information on the nutritional requirement to facilitate the farmers to understand the food and diet strategy during stress (Ding et al. 2017; Yong et al. 2020). In the biochemical process, glucose is considered an essential source of energy for many metabolic activities. Glucose is the result of carbohydrate metabolism that undergoes several chemical reactions involved in the Krebs cycle or the tricarboxylic acid cycle to generate the energy precursor in the form of Adenosine Tri-phosphate (Wang et al. 2016). The pentose phosphate pathway, which is negligible in fish, is the main carbohydrate metabolism pathway in decapod crustaceans during the ecdysis stage (Zhang et al. 2018). Research on carbohydrate metabolism in crustaceans still lacks compared to mammals or fish.

Environmental stress may have a significant impact on the energy balance of an organism due to the additional energy needed to recover and sustain homeostasis that may be confined to the systems involved in energy acquisition, conversion, and conservation (Schvezov et al. 2019). The potential energy cost of the stress response and homeostatic regulation against environmental disturbances have been addressed in several publications (Robles-Romo et al. 2016; Schvezov et al. 2019). Acclimation is a routine practice in the hatchery operations to mitigate the effects of various potential stressors that may inhibit the animals' physiological compensatory response (Sánchez-Paz et al. 2001; Ridgway et al. 2006; Lorenzon et al. 2007; Stoner 2012; Kumaresan et al. 2017) before it can be used for breeding purposes. Detailed haemolymph glucose patterns were obtained to establish a biomarker for the condition that would accurately reflect the state of this species's metabolism and energy reserves to compare cultivated prawns at the grow-out pond and prawns subjected to acclimation for short-term maintenance. Therefore, this study was performed to assess the effects of different handling conditions and moulting cycles on an animal's physiological state using glucose as a stress marker and evaluate the changes in lipid droplets in the hepatopancreas.

Materials and methods

Effects of handling stress and moulting cycle on energetic level of glucose, reproductive performance, and digestive capacity in pond-reared of female *M. rosenbergii* subjected to acclimation and *In Situ* sampling

The first experimental study was performed at the grow-out pond in Rembau, Negeri Sembilan, Malaysia (2.5905° N, 102.0930° E) with 26.9±2.1 °C water temperature and 7.43±0.14 pH value. The female prawns were divided into two experimental groups, whereby the first group (G1) was composed of cultivated prawns at the grow-out pond weighing 20.54±0.34 g captured using a fishing net. The prawns attached to the fishing net were sorted into a rectangular tray (4 m × 6 m × 4 m) afloat on the pond's surface. After

Table 1 Characteristics of moulting stages in giant freshwater prawn, *M. rosenbergii* as described by Kamaruding et al. (2018a)

Moulting stage	Exoskeleton texture	Epidermis structure	Seta Development
A	Soft	Light blue pigmentation, granular	Clear seta matrix
B	Hardened	Granular	The internal matrix developed within the seta
C0	Hard	Granular	The internal matrix cones develop within the seta
C1	Hard	Granular very dense	A matrix in the internal cones of the seta begins to retract
D0	Hard	Retraction of epidermis, pigmentation	Very fine structure of internal cones of seta
D1	Hard	New setal-forming regions appear in the epidermis surface	New seta begins to develop
D2	Hard	Developing new seta can be seen within the setal-forming regions in the epidermal surface	New seta form barbules
D3	Soft	Complete apolysis	Old setal organs disappear, new setae fold

sorting (maximum time took 2 h), randomly chosen prawns (N = 100) were placed in five containers containing 5 L of water (density 20–30 animals/container) equipped with portable aeration and transported to the sampling port. The dissolved oxygen level in the containers was recorded between 3.2 to 4 mg/mL. The prawns were sampled immediately after arriving at the sampling port. The second group of prawns (G2) consisted of short-term maintenance weighing 17.30±0.49 g was maintained under an open-shaded hatchery for 60 days. A total of 135 animals were housed individually separated by perforated plastics (3 animals per aquarium) in 45 glass aquaria (59 cm × 25 cm × 25 cm). The water temperature was set at 25.3±1.2°C and pH values ranging from 7.3±0.3. The water was changed twice a week with clean dechlorinated pipe water, and no food was offered during the 60-day experimental period. Newly moulted animals were identified each morning by the presence of exuviae besides the animal body, in which 5–7 animals were sampled each time. Briefly, animals were euthanised using the ice-narcotised method (placing the animal into a bucket of ice for 3 min) (Miranda-Anaya et al. 2003). About 100 µL of haemolymph was withdrawn from the pericardial cavity of the individual animal using a sterile 1 mL syringe fitted with a $27^{1/2G}$ needle into a 1 mL centrifuge tube. Both experimental groups (G1 and G2) were sampled according to the eight classifications of moulting stages as described by Kamaruding et al. (2018a). The prawns were classified according to the three overall stages, namely post-moult, inter-moult, and pre-moult. Each moulting stage was further divided into the following sub-stages that are post-moult (Sub-stages A and B), inter-moult (Sub-stages C0 and C1), and pre-moult (Sub-stages D0, D1, D2, and D3). The sub-stages of moulting were initially identified by gently touching the outer surface of the carapace and the hardness level of the rostrum. The carapace of the prawns at Sub-stage A is soft, and it hardens at Sub-stage B. As the moulting stage progresses during inter-moult and pre-moult, the carapace becomes fully hardened. A detailed analysis of setagenesis was performed under a stereomicroscope to identify the sub-stages between inter-moult and pre-moult stages (Olympus, Japan). Table 1 shows a description of each sub-stage of the post-moult, inter-moult, and pre-moult.

The haemolymph samples were kept at -80 °C for glucose analysis. Bodyweight (BW) was determined, ovary or hepatopancreas tissues were weighed, and the Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI) were then calculated using the following formulas (Zhang et al. 2007):

$$\text{GSI (\%)} = \frac{\text{Ovary weight (g)}}{\text{Body weight (g)}} \times 100$$

$$\text{HSI (\%)} = \frac{\text{Hepatopancreas weight (g)}}{\text{Body weight (g)}} \times 100$$

The glucose concentration of the haemolymph was quantified colourimetrically using a glucose-oxidase assay kit (Merck kGaA, Darmstadt, Germany). The analysis was performed according to the manufacturer's protocol. The procedure was conducted on a 96-well microplate consisting of a 2 µL sample, 48 µL assay

buffer, and 50 µL chromogenic reagent per well. The plate was incubated at 37 °C for 30 min to allow for the reaction to take place. The absorbance was recorded using a Multiskan Microplate Photometer (Thermo-Scientific, New Hampshire, United States) at 570 nm, and the concentrations were calculated from a standard solution of the substrates.

Effect of moulting cycle on the changes of lipid droplets in B cells of hepatopancreas and hepatosomatic index (HSI) in laboratory-maintained of female *M. rosenbergii*

Animals were acclimated under a laboratory condition for at least two weeks in a circular polyvinyl chloride (PVC) maturation tank with 5 tons of water capacity equipped with a centre drain to be used in the experiment. Horizontal strips of polyethylene were used as a shelter substrate for the newly moulted individuals. The quality of the water was monitored by the ammonium (≤ 0.03 ppm), nitrite (≤ 1 ppm), and nitrate (≤ 60 ppm) levels. The pH value was maintained within a range of 7.7±0.5. For each representative moulting stage, five individual prawns were collected and classified as Sub-stages A, B, C0, C1, D0, D1, D2, and D3. A small portion of hepatopancreas tissues was taken for histological investigation. The BW was recorded along with the ovary or hepatopancreas tissues, while the GSI and HSI were calculated using the formula as stated above (Zhang et al. 2007). Approximately 0.1 g middle portion of the left-side lobe of hepatopancreas tissues were fixed in 1 mL of Bouin's solution for 18 h. After fixation, the hepatopancreas tissues were cut into 1–2 mm^3 small blocks. The tissue blocks were then washed briefly with distilled water and 2–3 times in 70% ethanol until the yellow colour of the Bouin's solution was washed thoroughly. Dehydration, infiltration, and clearing of the hepatopancreas were carried out using an automatic tissue processor (Leica TP1020, Nussloch, Germany). The hepatopancreas tissues were then embedded into paraffin blocks using the paraffin embedding centre (Leica EG1160, Nussloch, Germany). The blocks were trimmed and cut to the appropriate sizes and sections at 5 µm thickness using a rotary microtome (Leica RM2235, Nussloch, Germany). The resulting ribbons were stretched onto 76 mm × 26 mm fine defrosted glass slides (Matsunami, Osaka, Japan) and coated with 1% acetic acid. The slides were then placed on a slide warmer at 37–40 °C to dry the excess acetic acid for at least 2 h to maximise the binding capacity of the tissue onto the slide surface. The slides were deparaffinised and hydrated using the standard protocol for histology. Next, the slides were stained using Haematoxylin and counterstain using Eosin-Y in 80% ethanol. After staining, the slides were observed under the microscope, and the images were captured using a digital image system connected to a computer (Olympus, Tokyo, Japan).

Data analysis and statistics

The normality of the data was determined by skewness and kurtosis z values within –1.96 and +1.96. As the data were normally distributed, a parametric analysis of variance was performed, followed by a comparison of means using Duncan's multiple range test. The data were considered significant at $P < 0.05$. Statistical analysis was performed using Statistical Package for Social Science (SPSS) Version 24. Pearson correlation was used to evaluate the significance of the relationship between BW, GSI, HSI, and haemolymph glucose concentration.

Results

Energetic level of glucose, reproductive performance, and digestive capacity in pond-reared of female *M. rosenbergii* subjected to acclimation and *In Situ* sampling

During the reproductive moulting cycle, the GSI of prawns under short-term maintenance showed the highest value at post-moult of A stage (10.52 ± 0.21%; $P < 0.05$; df =4; F value = 55.60) compared to the other stages (Fig. 1(A)). However, GSI showed the lowest values for prawns at B and C0 stages under short-term maintenance (0.37 ± 0.02% and 0.63 ± 0.05%, respectively) and grow-out pond (0.36 ± 0.03% and 0.60 ± 0.03%, respectively) compared to other moulting stages.

During the A stage of the reproductive moulting cycle, prawns that were kept in captivity showed lower HSI value (2.86 ± 0.14%; $P < 0.05$; df = 4; F value = 14.56) compared to C1 stage (4.57 ± 0.56%) (Fig.

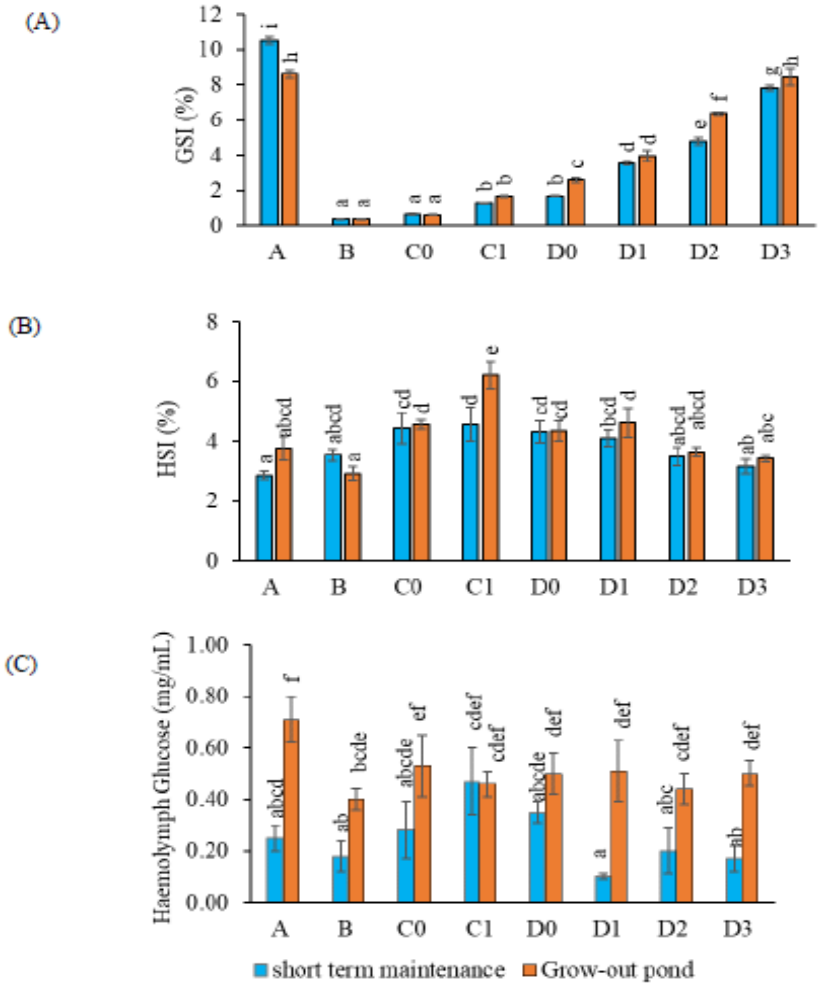

Fig. 1 Effect of moulting stage and handling stress on Gondosomatic Index (A), Hepatosomatic Index (B), and haemolymph glucose levels (C) of sub-adult female *M. rosenbergii* under short-term maintenance and grow-out pond during reproductive moulting cycle. Five animals were sampled for each point. Data are expressed as mean±S.E.M. Different letters in each point indicates significant difference (P <0.05) (N = 160, N represents total number of prawns sampled).

1(B)). No significant (P >0.05)differences were seen in prawns in both groups (in captivity and grow-out pond) during B, C0, D0, D1, and D2 stages with values ranging from 3.49 ± 0.31% to 4.61 ± 0.49%. Prawns that underwent C1 stage under grow-out pond had the highest GSI value (6.22 ± 0.46%; P <0.05; df = 4; F value = 33.52) compared to the other moulting stages under the short-term maintenance.

An abrupt increase of haemolymph glucose level was seen at A stage (0.71 ± 0.09 mg/mL) (Fig. 1(C)) in grow-out pond reared prawns compared to other moulting stages in short-term maintained prawns. However, there were no significant (P >0.05)differences in haemolymph glucose levels in short-term maintenance prawns at all moulting stages (A, B, C0, D0, D1, D2, and D3) with values ranging from 0.10 ± 0.01 to 0.47 ± 0.13 mg/mL. Similarly, the haemolymph glucose levels showed no significance (P >0.05)in grow-out pond reared prawns at all moulting stages (B, C0, D0, D1, D2, and D3) with values ranging from 0.40 ± 0.04 to 0.53 ± 0.12 mg/mL.

Unlike reproductive moulting cycle, short-term maintained prawns showed no significance (P >0.05) in GSI during non-reproductive moulting cycle throughout the moulting stages (A until D3 stages) with values ranging from 0.35 ± 0.02% to 0.45 ± 0.003% (Fig. 2(A)). In contrast, grow-out pond reared prawns showed a significant (P <0.05) difference in GSI value at A (0.43 ± 0.02%), B (0.39 ± 0.05%), and C0 (0.27 ± 0.01%) stages. However, there were no significant (P >0.05) differences at C1, D0, D1, D2, and D3 stages in grow-out pond reared prawns ranging from 0.30 ± 0.05% to 0.40±0.07%. In term of HSI, both short-term maintained (2.78 ± 0.44% to 4.30 ± 0.15%) and grow-out pond reared (4.00 ± 0.40% to 5.14

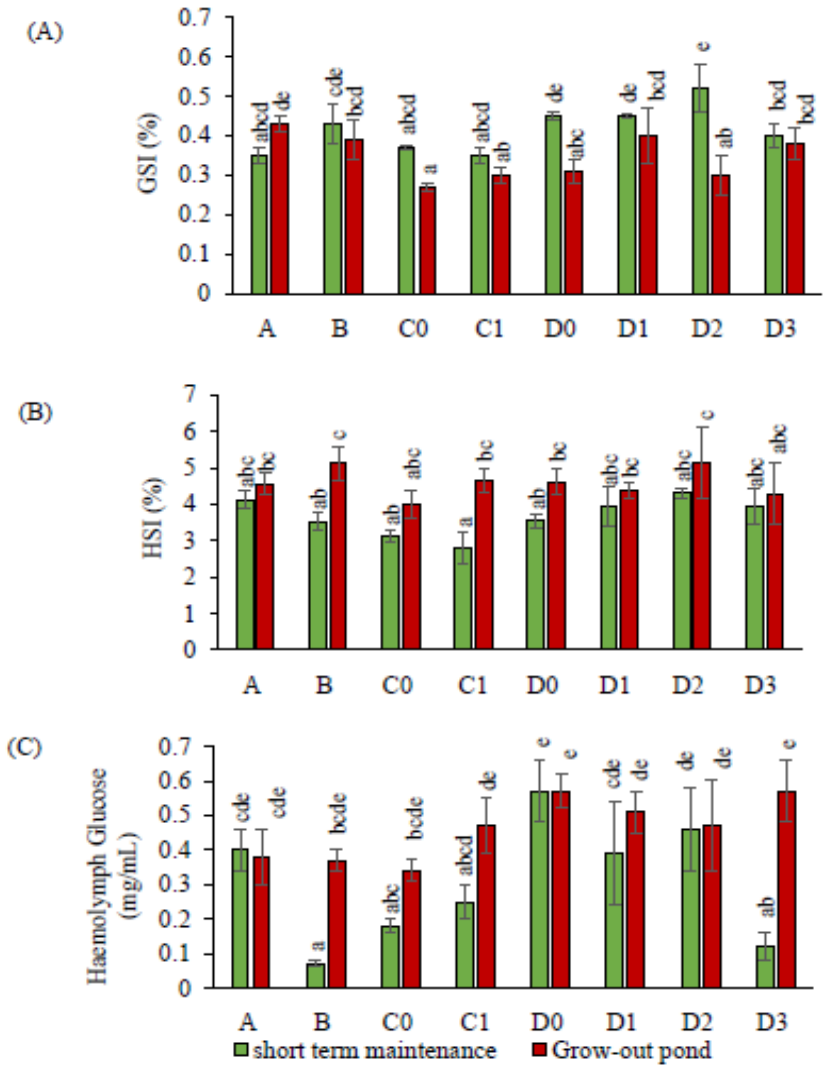

Fig. 2 Effect of moulting stage and handling stress on Gondosomatic Index (A), Hepatosomatic Index (B), and haemolymph glucose levels (C) of sub-adult female *M. rosenbergii* under short-term maintenance and grow-out pond during non-reproductive moulting cycle. Five animals were sampled for each point. Data are expressed as mean±S.E.M. Different letters in each point indicates significant difference (P <0.05) (N = 160, N represents total number of prawns sampled).

± 1.00%) prawns showed no significance (P >0.05) in all moulting stages (A until D3 stages) (Fig. 2(B)). Haemolymph glucose did not differ for all moulting stages in grow-out pond ranging from 0.34 ± 0.03 mg/mL to 0.57 ±0.09 mg/mL (Fig. 2(C)). However, under short-term maintenance, animals at B (0.07 ± 0.01 mg/mL) and D3 (0.12 ±0.04 mg/mL) stages showed a relatively lower haemolymph glucose than the other moulting stages.

Prawns underwent reproductive stage that was subjected to *in situ* samplings at grow-out pond showed higher glucose level in the haemolymph (0.60 ± 0.05 mg/mL; P <0.05; df = 4; F = 10.32) than animals subjected to short-term maintenance (Fig. 3). This indicates that glucose was utilised as the metabolic fuel in stressful conditions. In contrast, the non-reproductive stage showed no significance (P >0.05) in haemolymph glucose, indicating that glucose was utilised in the same manner in both groups with levels ranging from 0.30 ± 0.06 to 0.42 ± 0.03 mg/mL.

A negative relationship was also observed between GSI and HSI in animals under short-term maintenance (r = -0.53; P <0.05) (Table 2(a)). In contrast, animals that were maintained under the short-term showed a positive relationship between HSI and glucose (r = 0.35; P <0.05).

On the other hand, a negative relationship between BW and GSI (r = -0.40; P <0.05) was shown in the grow-out pond reared animals (Table 2(b)).

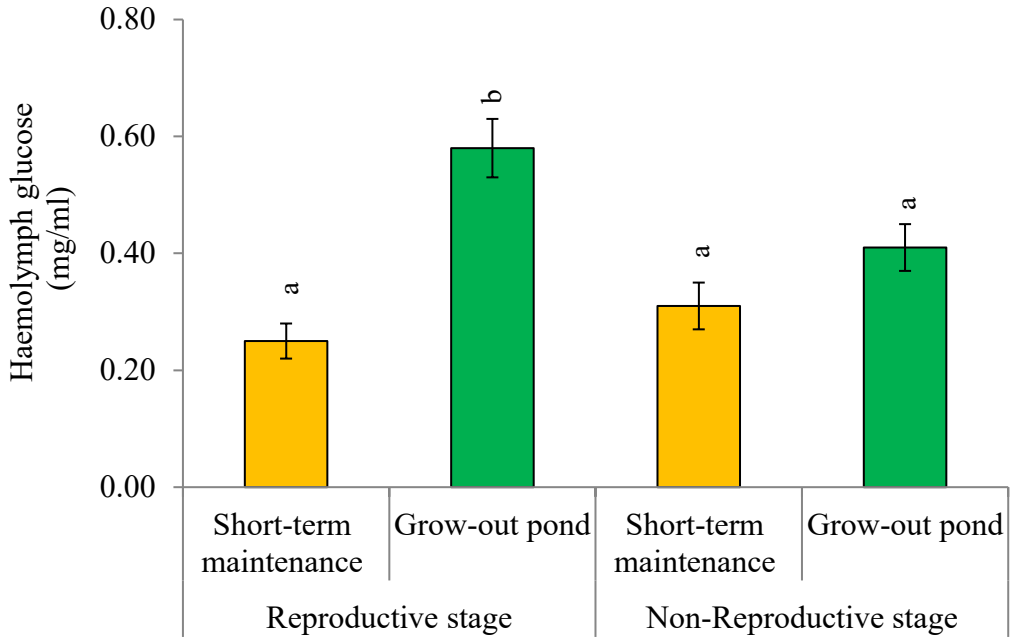

Fig. 3 Haemolymph glucose level (mean±S.E.M) in short-term maintenance and grow-out pond of female *M. rosenbergii* during the reproductive and non-reproductive stage.

Table 2 Correlations among a matrix of parameters in the female *M. rosenbergii* under short-term maintenance and grow-out pond during reproductive moulting cycle

(a)Short-term maintenance

	BW	GSI	HSI	Glucose
BW	1	-0.10 (40)	0.23 (40)	0.08 (39)
GSI	-	1	**-0.53 (40) ***	-0.20 (39)
HSI	-	-	1	**0.35 (39) ***
Glucose	-	-	-	1

*Pearson correlation was significantly different (P <0.05). The number in parentheses ( ) represents the number of prawns sampled.

(b)Grow-out Pond

	BW	GSI	HSI	Glucose
BW	1	**-0.40 (40) ***	0.30 (40)	-0.19 (40)
GSI	-	1	-0.26 (40)	0.27 (40)
HSI	-	-	1	0.11 (40)
Glucose	-	-	-	1

*Pearson correlation was significantly different (P <0.05). The number in parentheses ( ) represents the number of prawns sampled.

The correlations of matrix parameters in female *M. rosenbergii* under short-term maintenance and grow-out pond during the non-reproductive moulting cycle is shown in Table 3. There were positive relationships between GSI and HSI (r = 0.33; P <0.05) and GSI and glucose (r = 0.41; P <0.05) in short term-maintenance (Table 3(a)). In addition, the grow-out pond reared animals showed a positive relationship between HSI and glucose (r = 0.39; P <0.05)(Table 3(b)).

Changes of lipid droplets in B cells of hepatopancreas and hepatosomatic index (HSI)

Based on the histological section of the hepatopancreas in Fig. 4, the lipid droplets in the B cells were scarce either during post-moult (Sub-stages A and B) of the reproductive or non-reproductive moulting cycles. Conversely, a higher number of lipid droplets in B cells were observed in Sub-stages of C0 and C1 during

Table 3 Correlations of matrix parameters in female *M. rosenbergii* under short-term maintenance and grow-out pond during the non-reproductive moulting cycle

(a)Short-term maintenance

	BW	GSI	HSI	Glucose
BW	1	-0.06 (40)	0.25 (40)	0.20 (40)
GSI	-	1	**0.33 (40) ***	**0.41(40) ***
HSI	-	-	1	0.06 (40)
Glucose	-	-	-	1

*Pearson correlation was significantly different (P <0.05). The number in parentheses ( ) represents the number of prawns sampled.

(b)Grow-out pond

	BW	GSI	HSI	Glucose
BW	1	-0.08 (40)	0.21 (40)	0.22 (40)
GSI	-	1	-0.07 (40)	0.03 (40)
HSI	-	-	1	**0.39 (40)***
Glucose	-	-	-	1

*Pearson correlation was significantly different (P <0.05). The number in parentheses ( ) represents the number of prawns sampled.

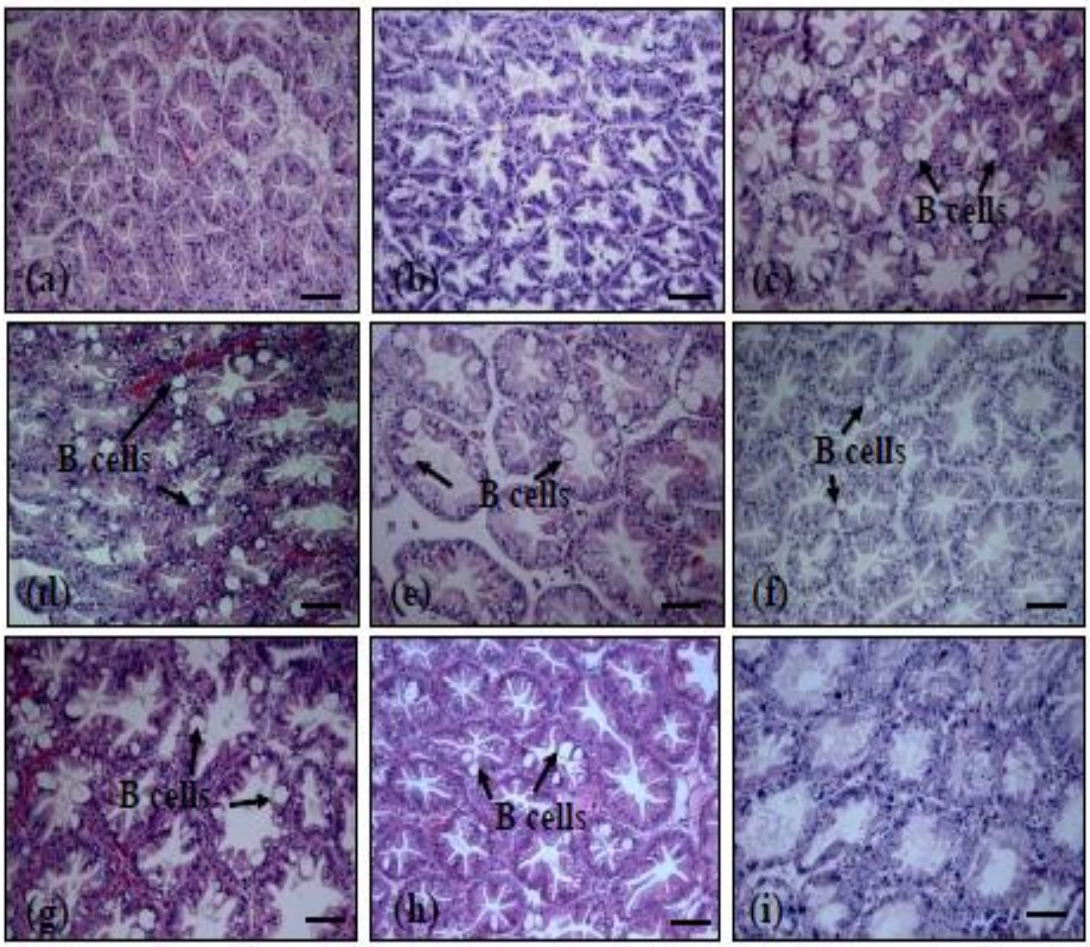

Fig. 4 Histological section of hepatopancreas (H&E staining) during reproductive and non-reproductive moulting cycles in the female *M. rosenbergii* (magnification 100X) (a) Sub-stage A; a scarce number of B cells (b) B stage; scarce number of B cells (c) Sub-stage C0; high number of B cells (d) Sub-stage C1; a high number of B cells (e) Sub-stage D0; low number of B cells (f) Sub-stage D1; low number of B cells (g) Sub-stage D2; low number of B cells (h) Sub-stage D3; low number of B cells and (i) Non-reproductive moulting cycle; a scarce number of B cells. Arrow indicates lipid droplets of B cells. Scale bar: 100 μm.

Table 4 Changes in HSI during reproductive and non-reproductive moulting cycles in the female *M. rosenbergii*. Different letters in indicate significant difference (P <0.05) (N = 73, N represents total number of prawns sampled).

Moulting stage	HSI (%)		GSI (%)	
	Reproductive moulting cycle	Non-reproductive moulting cycle	Reproductive moulting cycle	Non-reproductive moulting cycle
A	3.82±0.33^{a}	4.65±0.41^{a}	9.58±0.41^{f}	0.48±0.02^{a}
B	3.78±0.39^{a}	-	0.54±0.06^{a}	-
C0	4.35±0.28^{a}	5.15±0.23^{a}	0.57±0.02^{a}	0.46±0.04^{a}
C1	6.18±0.81^{b}	3.80±0.62^{a}	1.21±0.14^{ab}	0.44±0.04^{a}
D0	5.04±0.44^{ab}	4.91±0.42^{a}	1.69±0.18^{bc}	0.43±0.04^{a}
D1	5.07±0.51^{ab}	5.15±0.16^{a}	2.64±0.35^{c}	0.42±0.06^{a}
D2	4.89±0.29^{ab}	4.74±0.44^{a}	4.29±0.19^{d}	0.51±0.11^{a}
D3	5.37±0.71^{ab}	4.31±0.72^{a}	6.28±0.83^{e}	0.36±0.09^{a}

inter-moult and reduced when approaching late pre-moult (Sub-stage D3) of the reproductive moulting cycle.

HSI values were not significantly different (P >0.05) during the non-reproductive moulting cycle in which the range value was between 3.80 ± 0.62 to 5.15 ± 0.23% (Table 4). Conversely, HSI shows the highest significant value at Sub-stage C1 during the reproductive moulting cycle (6.18 ± 0.81%; P <0.05; df = 4; F = 20.6) compared to post-moult (Sub-stages A and B; 3.82 ± 0.33 and 3.78 ± 0.39%, respectively) and inter-moult Sub-stage C0 (4.35 ± 0.28%). The HSI values during early and late pre-moult stages were in the range of 4.89 ± 0.29% to 5.37 ± 0.71%.

Discussion

Moulting cycle affects ovarian development in crustaceans. Results in this study showed a synchronise ovarian development and moulting stages in *M. rosenbergii.* Similar findings were also reported by Okumura and Aida (2000) and Kamaruding et al. (2017). Both groups showed a similar pattern in GSI and HSI values, in which these values were consistent throughout the non-reproductive moulting cycle. The moulting cycle of the Palaemonidae species, such as *M. rosenbergii,* can be divided into two modes, namely reproductive and non-reproductive moulting cycles. The reproductive moulting cycle is defined as a period when the shedding of the exoskeleton occurs in coordination with ovarian development (Mrak et al. 2017). Generally, two moulting cycles are needed to complete the ovarian development cycle. In the first moult, the ovary develops to become completely vitellogenic at ecdysis, and in the second moult, mating and spawning occur. During post- and inter-moult stages, all unfertilised eggs were released from the female's body (Chang and Mykles 2011). In contrast, the non-reproductive moulting cycle is a period whereby the shedding of the exoskeleton is not accompanied by ovarian development, but this cycle represents only somatic growth (Kilada and Driscoll 2017; Mrak et al. 2017). Several extrinsic factors affect ecdysis and growth in crustaceans, including temperature, light and photoperiod, water alkalinity, size and sex, season, density and space, nutrition and food, habitat, chemical contaminants, microplastics, handling, and transport stressors (Dise and Goldina 2017; Espinosa-Chaurand et al. 2017; Santos-Romero et al. 2017; Yuan et al. 2017; Kamaruding et al. 2018b; Day et al. 2019; Mota et al. 2021). The intrinsic factors that affect the ecdysis and growth of crustaceans include developmental stage, reproductive maturity, the status of limb regeneration, and hormonal control (Sugumar et al. 2013; Subramoniam 2017).

Glucose is a molecule that has a significant role in the energy metabolism of crustaceans. Variations in glucose levels measured in the haemolymph are related to the quantity and quality of the carbohydrates contained in the diet (Rosas et al. 2000; Rodríguez-Viera et al. 2014; Ding et al. 2017). In addition, Wells and Pankhurst (1999) reported a positive correlation of handling and confinement stress with glucose in the rainbow trout of *Oncorhynchus mykiss*. In this study, excessive exposure to heat and low dissolved oxygen (<3.2 mg/mL) were the main causative effects contributing to the elevated level of glucose in the grow-out pond reared animals. During stress, most of the energy was channelled to withstand a chronic exposure towards stressors, and lesser energy reserves were allocated for reproduction and growth (Baliña et al. 2018; Borges et al. 2018; Yu et al. 2018; Glazier et al. 2020). Similarly, in this study, more energy was

directed towards prawns that went through the reproductive cycle in the grow-out ponds (unacclimated) despite being subjected to excessive heat and low dissolved oxygen compared to the non-reproductive prawns in both the acclimated and grow-out (unacclimated) conditions.

In principle, B cells are secretory and absorptive cells with a high proportion of lipid droplets and glycogen. Additionally, B cells are also granulated with metal ions. These cells undergo ultrastructural alterations during fasting and moulting, as well as when metal-contaminated food is provided to animals (Vogt 2019; Huang et al. 2020). In this study, the numbers of lipid droplets within the B cells were scarce during post-moult (Sub-stages A and B). Furthermore, the scarcity of B cells localised to the proximal end of tubules during the post-moult stage (Sub-stages A and B) was consistent with another study by Al-Mohanna and Nott (1989) hence implying that this state is related to the post-moult stage during the starving condition. Zilli et al. (2003) further discovered that the number of B cells in Kuruma Prawn (*Marsupenaeus japonicus*) were lower in the fasting animals (for 48 h) compared to the customarily fed animals. A few hours after exuviation, as the animals entered the post-moult stage, the response to food intake was very minimal, and the prawns were inactive during feeding. This is a possible explanation for the scarcity of B cells during post-moult and may be related to the function of B cells as the main site for the synthesis of digestive enzymes (Al-Mohanna and Nott 1986; Vogt 1993; Sánchez-Paz et al. 2007; Vogt 2019; Huang et al. 2020). Similar findings were also reported by Thongrod et al. (2018), whereby reduced lipid droplets were recorded in the hepatopancreas during fasting and were restored after re-feeding. Another possible role of lipids is that they are stored in hepatopancreatic cells before moulting to support the formation of the new exoskeleton (Travis 1955; Alikhan 1972; Lešer et al. 2008; Tian et al. 2012). Meanwhile, the number of lipid droplets in B cells during the inter-moult stage (Sub-stages C0 and C1) was higher than in the pre-moult stage. The high number of lipid droplets in B cells could be linked to the high rate of synthesis and release of digestive and antioxidant enzymes. The release of the compounds accelerates the mobilisation of nutrients to the hepatopancreas tubules thereby supplying energy to accommodate the demands of activity (Verri et al. 2001; Li et al. 2008).

Conclusions

Animals collected at the grow-out pond utilised more energy than the animals acclimated under the short-term as evidenced by an increase in glucose levels. This was linked to handling and environmental stresses. The moulting cycle affected the number of lipid droplets in the hepatopancreatic B cells. The lipid droplets during the inter-moult stage were greater than the pre-moult stage hence indicating a significant mobilisation of resources in hepatopancreas tubules for energy-generating to accommodate vigorous metabolic activity before moulting. This study recommends that crustacean aquaculture producers should consider acclimation during the rearing of broodstocks for spawning induction to minimise the amount of energy used for stress tolerance while maximising growth capacity and reproductive success.

Acknowledgments The authors gratefully acknowledged the staff at PELADANG Tanjong Ipoh, Negeri Sembilan, Malaysia and aquaculture farmers in Negeri Sembilan who helped during the experiment and providing the live prawn samples. We also would like to thank staff at Physiology laboratory, Institute of Tropical Aquaculture and Institute of Marine Biotechnology for technical assistance. This study was the first author's Ph.D. research work, which was supported by the Ministry of Education of Malaysia, under the IPTA Academic Training Scheme.

Conflicts of interest The authors declare that they have no conflict of interest.

References

Alikhan MA (1972) Changes in the hepatopancreas metabolic reserves of *Porcellio laevis* Latrielle during starvation and the moult cycle. Am Midl Nat 87:503-514. https://doi.org/10.2307/2423579

Al-Mohanna SY, Nott JA (1986) B-cells and digestion in the hepatopancreas of *Penaeus semisulcatus* (Crustacea: Decapoda). J Mar Biol Assoc 66:403-414. https://doi.org/10.1017/S0025315400043034

Al-Mohanna SY, Nott JA (1989) Functional cytology of the hepatopancreas of *Penaeus semisulcatus* (Crustacea: Decapoda) during the moult cycle. Mar Biol 101:535-544. https://doi.org/10.1007/BF00541656

Baliña S, Temperoni B, Greco LSL, Tropea C (2018) Losing reproduction: effect of high temperature on female biochemical composition and egg quality in a freshwater crustacean with direct development, the Red Cherry Shrimp, *Neocaridina davidi* (Decapoda, Atyidae). Biol Bull 234(3):139-151. https://doi.org/10.1086/698266

Banu R, Christianus A (2016) Giant freshwater prawn, *Macrobrachium rosenbergii* farming: A review on its current status and

prospective in Malaysia. J Aquac Res Development 7 (4): 1-5. https://doi.org/10.4172/2155-9546.1000423

Bonilla-Gomez JL, Chiappa-Carrara X, Galindo C, Jeronimo G, Cuzon G, Gaxiola G (2012) Physiological and biochemical changes of wild and cultivated juvenile Pink Shrimp *Farfantepenaeus duorarum* (Crustacea: Penaeidae) during moult cycle. J Crustacean Biol 32(4):597-606. https://doi.org/10.1163/193724012X630679

Borges FO, Sampaio E, Figueiredo C, Rosa R, Grilo TF (2018) Hypercapnia-induced disruption of long-distance mate-detection and reduction of energy expenditure in a coastal keystone crustacean. Physiol Behav 195:69-75. https://doi.org/10.1016/j.physbeh.2018.07.023

Chang ES, Mykles DL (2011) Regulation of crustacean moulting: A review and our perspectives. Gen Comp Endocrinol 172(3): 323-330. https://doi.org/10.1016/j.ygcen.2011.04.003

Cook KV, Reid AJ, Patterson DA, Robinson KA, Chapman JM, Hinch SG, Cooke SJ (2019) A synthesis to understand responses to capture stressors among fish discarded from commercial fisheries and options for mitigating their severity. Fish Fisheries 20:25-43. https://doi.org/10.1111/faf.12322

Day RD, Fitzgibbon QP, Gardner C (2019) The impact of holding stressors on the immune function and haemolymph biochemistry of Southern Rock Lobsters (*Jasus edwardsii*). Fish Shellfish Immunol 89:660-671. https://doi.org/10.1016/j.fsi.2019.03.043

Ding L, Fu H, Hou Y, Jin M, Sun P, Zhou Q (2017) Effects of starvation and feeding on blood chemistry, fatty acid composition, expression of vitellogenin and fatty acid-binding protein genes in female Swimming Crab, *Portunus trituberculatus* broodstock. Fish Sci 83: 455-464. https://doi.org/10.1007/s12562-017-1075-3

Ding ZL, Kong YQ, Li JF, Cao F, Zhang YX, Du ZY, Ye JY (2017) Growth and metabolic responses of juvenile *Macrobrachium nipponense* to different dietary carbohydrate levels. Aquac Nutr 23(5):1136-1144. https://doi.org/10.1111/anu.12482

Dise K, Goldina A (2017) Social instability stimulates moulting in *Orconectes obscurus*. Bios 88(1):46-54. https://doi.org/10.1893/BIOS-D-15-00006.1

Espinosa-Chaurand D, Vega-Villasante F, Carrillo-Farnés O, Nolasco-Soria H (2017) Effect of circadian rhythm, photoperiod, and moult cycle on digestive enzymatic activity of *Macrobrachium tenellum* juveniles. Aquacult 479:225-232. https://doi.org/10.1016/j.aquaculture.2017.05.029

FAO (2009) The state of world fisheries and aquaculture 2008. FAO Fisheries Department, Rome, Italy. pp. 162

Glazier DS, Borrelli JJ, Hoffman CL (2020) Effects of fish predators on the mass-related energetics of a keystone freshwater crustacean. Biol 9(3):1-34. https://doi.org/10.3390/biology9030040

Huang X, Feng Y, Duan J, Xiong G, Fan W, Liu S, Zhong L, Wang K, Geng Y, Ouyang P, Chen D, Yang S, Yin L (2020) Anti-starvation strategies of *Eriocheir Sinensis*: regulatory networks under hepatopancreas consumption. Oxid Med Cell Longev 6085343:1-16. https://doi.org/10.1155/2020/6085343

Kamaruding NA, Ismail N, Jasmani S, Wilder MN, Ikhwanuddin M (2017) Dynamics of glucose in the haemolymph of female Giant Freshwater Prawn, *Macrobrachium rosenbergii* influences reproductive and non-reproductive moulting cycles. Aquac Res 48:3505-3514. https://doi.org/10.1111/are.13176

Kamaruding NA, Ismail N, Ikhwanuddin M (2018a) Characterization of moulting stages in the Giant Freshwater Prawn, *Macrobrachium rosenbergii* using setagenesis of pleopod. Songklanakarin J Sci Technol 40(2): 1-5.

Kamaruding NA, Ismail N, Ikhwanuddin M (2018b) Physiological effect of eyestalk ablation on nutrient utilization and plasma protein expression in the female Giant Freshwater Prawn (*Macrobrachium rosenbergii*) during different moulting cycles. J Shellfish Res 37(5):1-8. https://doi.org/10.2983/035.037.0523

Kilada R, Driscoll JG (2017) Age determination in crustaceans: A review. Hydrobiol 799:21-36. https://doi.org/10.1007/s10750-017-3233-0

Kumaresan V, Palanisamy R, Pasupuleti M, Arockiaraj J (2017) Impacts of environmental and biological stressors on immune system of *Macrobrachium rosenbergii*. Rev Aquacult 9(3): 283-307. https://doi.org/10.1111/raq.12139

Lešer V, Drobne D, Vilhar B, Kladnik A, Žnidaršič N, Štrus J (2008) Epithelial thickness and lipid droplets in the hepatopancreas of *Porcellio scaber* (Crustacea: Isopoda) in different physiological conditions. Zool 111(6):419-432. https://doi.org/10.1016/j.zool.2007.10.007

Li E, Chen L, Zeng C, Yu N, Xiong Z, Chen X, Qin JG (2008) Comparison of digestive and antioxidant enzymes activities, haemolymph oxyhaemocyanin contents and hepatopancreas histology of White Shrimp, *Litopenaeus vannamei* at various salinities. Aquacult 274:80-86. https://doi.org/10.1016/j.aquaculture.2007.11.001

Lorenzon S, Giulianini PG, Martinis M, Ferrero EA (2007) Stress effect of different temperatures and air exposure during transport on physiological profiles in the American Lobster *Homarus americanus*. Comp Biochem Physiol A Mol Integr Physiol 147(1): 94-102. https://doi.org/10.1016/j.cbpa.2006.11.028

Lorenzon S, Martins M, Borne D, Ferrero EA (2013) Haemolymph parameters as physiological biomarkers for monitoring the effects of fishing and commercial maintenance methods in *Squilla mantis* (Crustacea, Stomatopoda). Fish Res 137:9-17. https://doi.org/10.1016/j.fishres.2012.08.015

Lund HS, Wang T, Chang ES, Pedersen LF, Taylor EW, Pedersen PB, McKenzie DJ (2009) Recovery by the Norway Lobster *Nephrops norvegicus* (L.) from the physiological stresses of trawling: influence of season and live storage position. J Exp Mar Biol Ecol 373:124-132. https://doi.org/10.1016/j.jembe.2009.04.004

Mercier L, Palacios E, Campa-Córdova AI, Tovar-Ramirez D, Hernández-Herrera R, Racotta IS (2006) Metabolic and immune responses in Pacific Whiteleg Shrimp *Litopenaeus vannamei* exposed to a repeated handling stress. Aquacult 258:633-640. https://doi.org/10.1016/j.aquaculture.2006.04.036

Miranda-Anaya M, Barrera-Mera B, Ramírez-Lomelí E (2003) Circadian locomotor activity rhythm in the freshwater crab, *Pseudothelphusa americana* (De Saussure, 1857): effect of eyestalk ablation. Biol Rhythm Res 34(2):167–176. https://doi:10.1076/brhm.34.2.167.14486

Mota VC, Siikavuopio SI, James P (2021) Physiological responses to live air transport of Red King Crab (*Paralithodes camtschaticus*). Fisheries Res 237:1-6. https://doi.org/10.1016/j.fishres.2021.105882

Mrak P, Bogataj U, Štrus J, Žnidaršič N (2017) Cuticle morphogenesis in crustacean embryonic and post-embryonic stages. Arthropod Struct Dev 46(1):77-95. https://doi.org/10.1016/j.asd.2016.11.001

Okumura T, Aida K (2000) Haemolymph vitellogenin levels and ovarian development during the reproductive and non-reproductive

moult cycles in the Giant Freshwater Prawn, *Macrobrachium rosenbergii*. Fish Sci 66:678–685. https://doi.org/10.1046/j.1444-2906.2000.00108.x

Ridgway ID, Taylor AC, Atkinson RJA, Chang ES, Neil DM (2006) Impact of capture method and trawl duration on the health status of the Norway Lobster, *Nephrops norvegicus*. J Exp Mar Biol Ecol 339:135-147. https://doi.org/10.1016/j.jembe.2006.07.008

Robles-Romo A, Zenteno-Savin T, Racotta IS (2016) Bioenergetic status and oxidative stress during escape response until exhaustion in Whiteleg Shrimp, *Litopenaeus vannamei*. J Exp Mar Biol Ecol 478:16-23. https://doi.org/10.1016/j.jembe.2016.01.016

Rodríguez-Viera L, Perera E, Casuso A, Perdomo-Morales R, Gutierrez O, Scull I, Carrillo O, Martos-Sitcha JA, García-Galano T, Mancera JM (2014) A holistic view of dietary carbohydrate utilization in lobster: digestion, postprandial nutrient flux, and metabolism. Plos One 9(9):1-17. https://doi.org/10.1371/journal.pone.0108875

Rosas C, Cuzon G, Arena L, 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(2):181–198. https://doi.org/10.1016/S0022-0981(00)00184-2

Sánchez-Paz A, Pascual C, Sánchez A, Vargas-Albores F, Le Moullac G, Rosas C (2001) Haemolymph metabolic variables and immune response in *Litopenaeus setiferus* adult males: the effect of acclimation. Aquacult 198:13-28. https://doi.org/10.1016/S0044-8486(00)00576-7

Sánchez-Paz A, García-Carreño F, Hernández-López J, Muhlia-Almazán A, Yepiz-Plascencia G (2007) Effect of short-term starvation on hepatopancreas and plasma energy reserves of the Pacific White Shrimp (*Litopenaeus vannamei*). J Exp Mar Biol Ecol 340:184–193. https://doi.org/10.1016/j.ibmb.2006.01.002

Santos-Romero RDL, García-Guerrero M, Vega-Villasante F, Cortés-Jacinto E, Nolasco-Soria H (2017) Effect of photoperiod and temperature on growth and activity of digestive enzymes in juveniles of the Longarm River Shrimp *Macrobrachium tenellum* (Smith, 1871)(Caridea: Palaemonidae). J Crustacean Biol 37(4):445-452. https://doi.org/10.1093/jcbiol/rux055

Schvezov N, Lourich GA, Tapella F, Gowland-Sainz M, Romero MC (2019) Effect of the temperature of air exposure on the oxidative stress status of commercial male Southern King Crab, *Lithodes santolla*. Fish Res 212:188-195. https://doi.org/10.1016/j.fishres.2018.12.020

Sokolova IM (2013) Energy-limited tolerance to stress a conceptual framework to integrate the effects of multiple stressors. Integr Comp Biol 53(4):597-608. https://doi.org/10.1093/icb/ict028

Stoner AW (2012) Assessing stress and predicting mortality in economically significant crustaceans. Rev Fish Sci 20(3):111-135. https://doi.org/10.1080/10641262.2012.689025

Subramoniam T (2017) Steroidal control of vitellogenesis in crustacea: a new understanding for improving shrimp hatchery production. Proceedings of the Indian Nat Sci Acad 83:595–610. https://doi.org/10.16943/ptinsa/2017/48969

Sugumar V, Vijayalakshmi G, Saranya K (2013) Moult 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. https://doi.org/10.1007/s00343-017-5337-9

Thongrod S, Wanichanon C, Kankuan W, Siangcham T, Phadngam S, Morani F, Isidoro C, Sobhon P (2018) Autophagy-associated shrinkage of the hepatopancreas in fasting male *Macrobrachium rosenbergii* is rescued by neuropeptide F. Front Physiol 9:1-9. https://doi.org/10.3389/fphys.2018.00613

Tian Z, Kang X, Mu S (2012) The moult stages and the hepatopancreas contents of lipids, glycogen and selected inorganic elements during the moult cycle of the Chinese Mitten Crab *Eriocheir sinensis*. Fish Sci 78:67–74. https://doi.org/10.1007/s12562-011-0426-8

Travis OF (1955) The moulting cycle of the Spiny Lobster, *Panulirus argus* latreille. ii. pre-ecdysial histological and histochemical changes in the hepatopancreas and integumental tissues. Biol Bull 108(1):88-112. https://doi.org/10.2307/1538400

Troha K, Ayres JS (2020) Metabolic adaptations to infections at the organismal level. Trends Immunol 41(2):113-125. https://doi.org/10.1016/j.it.2019.12.001

Verri T, Mandal A, Zilli L, Bossa D, Mandal PK, Ingrosso L, Zonno V, Vilella S, Ahearn GA, Storelli C (2001) D-glucose transport in decapod crustacean hepatopancreas. Comp Biochem Phys A 130(3):585-606. https://doi.org/10.1016/S1095-6433(01)00434-2

Vogt G (1993) Differentiation of B cells in the hepatopancreas of the prawn *Penaeus monodon*. Acta Zool 74(1):51-60. https://doi.org/10.1111/j.1463-6395.1993.tb01220.x

Vogt G (2019) Functional cytology of the hepatopancreas of decapod crustaceans. J Morphol 280(9):1405-1444. https://doi.org/10.1002/jmor.21040

Wang JT, Han T, Li XY, Hu SX, Jiang YD, Wang CL (2016) Effects of dietary phosphatidylcholine (PC) levels on the growth, moult performance and fatty acid composition of juvenile Swimming Crab, *Portunus tribuberculatus*. Anim Feed Sci Tech 216:225-233. https://doi.org/10.1016/j.anifeedsci.2016.03.023

Wells RMG, Pankhurst WN (1999) Evaluation of simple instruments for the measurement of blood glucose and lactate, and plasma protein as stress indicators in fish. J World Aquacult Soc 30(2):276–284. https://doi.org/10.1111/j.1749-7345.1999.tb00876.x

Yong ASK, Mok WY, Tamrin MLM, Shapawi R, Kim YS (2020) Effects of dietary nucleotides on growth, survival and metabolic response in Whiteleg Shrimp*, Litopenaeus vannamei* against ammonia stress condition. Aquac Res 51(6):2252-2260. https://doi.org/10.1111/are.14570

Yu N, Ding QQ, Li E, Qin JG, Chen L, Wang X (2018) Growth, energy metabolism and transcriptomic responses in Chinese Mitten Crab (*Eriocheir sinensis*) to benzo[α]pyrene (BaP) toxicity. Aquat Toxicol 203:150-158. https://doi.org/10.1016/j.aquatox.2018.08.014

Yuan Q, Wang Q, Zhong T, Li Z, Liu J (2017) Effects of water temperature on growth, feeding and moulting of juvenile Chinese Mitten Crab, *Eriocheir sinensis*. Aquacult 468:169-174. https://doi.org/10.1016/j.aquaculture.2016.10.007

Zhang IL, Zuo ZH, Chen YX, Zhao Y, Hu S, Wang CG (2007) Effect of tributyltin on the development of ovary in female Cuvier (*Sebastiscus marmoratus*). Aquac Toxicol 83:174–179. https://doi.org/10.1016/j.aquatox.2007.03.018

Zhang Y, Qin C, Yang L, Lu R, Zhao X, Nie G (2018) A comparative genomics study of carbohydrate / glucose metabolic genes: from fish to mammals. BMC genomics 19(246): 1-14. https://doi.org/10.1186/s12864-018-4647-4

Zilli L, Schaivone R, Scordella G, Zonno V, Verri T, Storelli C, Viella S (2003) Changes in cell type composition and enzymatic activities in the hepatopancreas of *Marsupenaeus japonicus* during the moulting cycle. J Comp Physiol B. 173:355-363. https://doi.org/10.1007/s00360-003-0348