



Research article

Low water pH depressed growth and early development of giant freshwater prawn *Macrobrachium rosenbergii* larvae

Hon Jung Liew^{a,i,j,*}, Sharifah Rahmah^{a,b}, Pei Wen Tang^{a,b}, Khor Waiho^{a,j,k},
Hanafiah Fazhan^{a,j}, Nadiyah Wan Rasdi^{b,e}, Siti Izzah Athirah Hamin^{a,b}, Suhairi Mazelan^{a,j},
Sabri Muda^{a,j}, Leong-Seng Lim^f, Young-Mao Chen^{g,h}, Yu Mei Changⁱ, Li Qun Liangⁱ,
Mazlan Abd. Ghaffar^{a,c,d}

^a Higher Institution Centre of Excellence (HICOE), Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, Malaysia

^b Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, Malaysia

^c Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, Malaysia

^d Institute of Marine Biotechnology, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, Malaysia

^e Institute of Tropical Biodiversity and Sustainable Development, Universiti Malaysia Terengganu, Kuala Nerus, Terengganu, Malaysia

^f Borneo Marine Research Institute, Universiti Malaysia Sabah, Kota Kinabalu, Sabah, Malaysia

^g Bachelor Degree Program in Marine Biotechnology, College of Life Science, National Taiwan Ocean University, Keelung, Taiwan

^h Center of Excellence for the Oceans, National Taiwan Ocean University, Keelung, Taiwan

ⁱ Heilongjiang River Fisheries Research Institute, Chinese Academy of Fishery Science, Harbin, China

^j STU-UMT Joint Shellfish Research Laboratory, Shantou University, Shantou, Guangdong, China

^k Center for Chemical Biology, Universiti Sains Malaysia, Minden, Malaysia

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ABSTRACT

Macrobrachium rosenbergii is one of the shellfish species with high aquaculture value due to its increasing market demand. However, the comparatively low production volume compared to demand coupled with the rapid decline of the natural environment, consequently, drives the potential depletion of the wild population. The decrease in water pH related to anthropogenic pollution is one of the most critical factors affecting the early life performances of *M. rosenbergii*. Therefore, this study was designed to examine the effect of low water pH on feeding, growth and development of *M. rosenbergii* early life stages. Experimental water pH was set as neutral (7.7 ± 0.4); mild-acidic (6.4 ± 0.5) and acidic (5.4 ± 0.2) with triplication at a stocking density of 2 larvae/L for 30 days. As expected, *M. rosenbergii* larvae were highly sensitive to acidic pH with no larvae survived beyond 48 h of exposure. Feeding, survival and growth of larvae were adversely affected by mild-acidic pH exposure as compared to neutral pH. Larvae exposed to mild-acidic water pH experienced a prolonged larval period and only metamorphosed to the post-larval stage at day-30. Whilst under neutral water pH, larval that metamorphosed to post-larval was first observed on day-23. The negative impact of decreased pH, even in mild-acidic pH exposure, on the feeding, survival, growth and development of *M. rosenbergii* larvae highlights the urgency of periodic pH monitoring during *M. rosenbergii* larviculture.

1. Introduction

Macrobrachium rosenbergii or commonly known as giant freshwater prawn is one of the most popular freshwater crustacean species owing to its high market demands and commercial values throughout the Asia Pacific region (Setiadi and Taufik, 2018). Increment of *M. rosenbergii* farming and production has been observed in the past decades and yet, the production is still unable to fulfil market needs due to the escalating

increase in demand (New and Nair, 2012; Liew et al., 2021). In nature, *M. rosenbergii* inhabits most of the inland freshwater areas such as lakes, rivers, swamps, irrigation canals, ponds as well as estuarine areas (Davassi, 2011). *M. rosenbergii* females migrate to estuaries for spawning, where the larvae grow in brackish water, until they metamorphose into the post-larvae stage (Liew et al., 2021). Thereafter, the post-larvae will migrate to upstream and continue to grow to reach the maturation stage in the freshwater environment. Until mating season resume,

* Corresponding author.

E-mail address: honjung@umt.edu.my (H.J. Liew).

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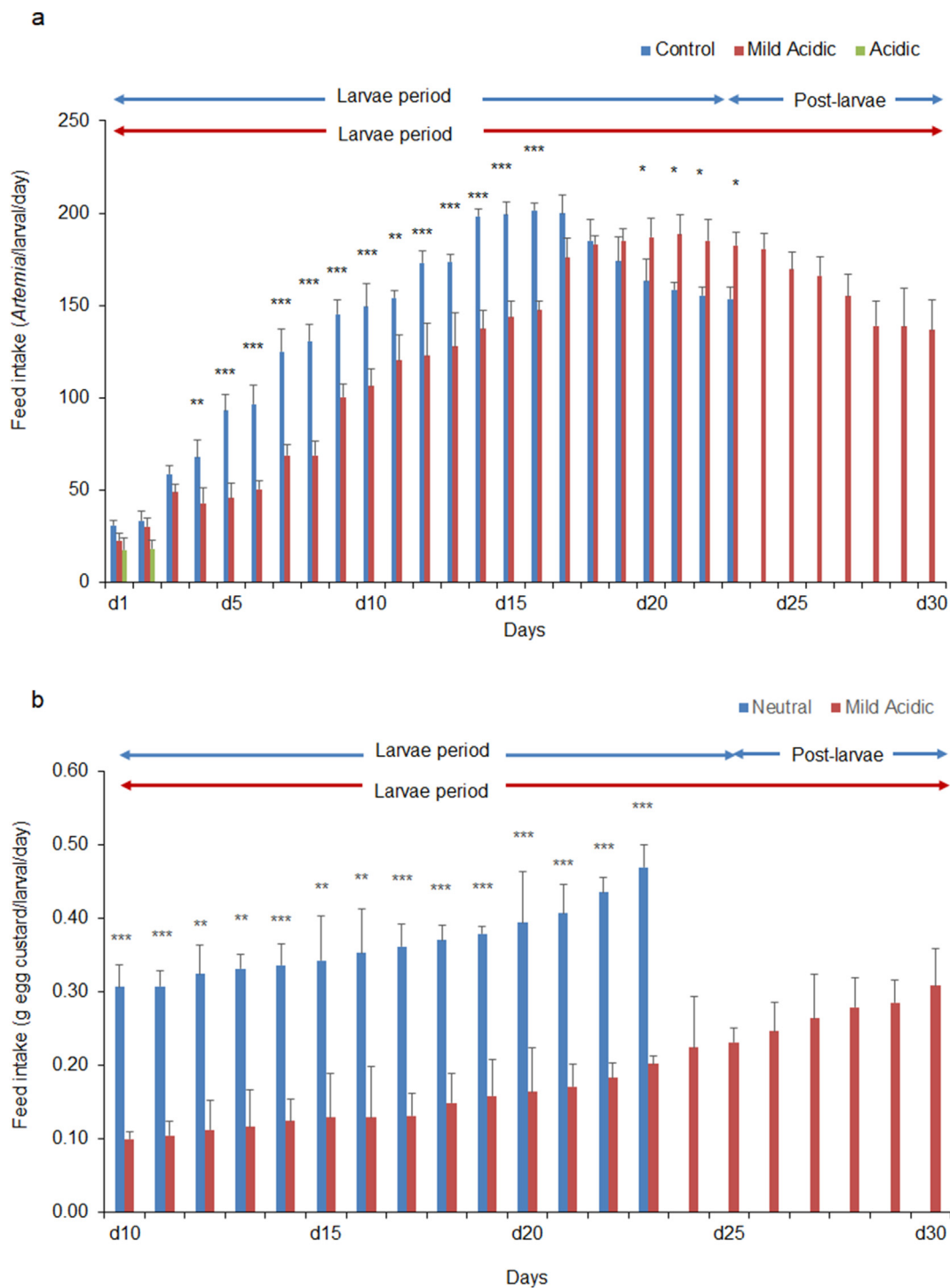


Figure 1. (a) Feed intake (*Artemia*/larval/day) of *M. rosenbergii* larvae exposed to neutral pH for 23 days (blue bars), mild-acidic pH for 30 days (red bars) and acidic pH for 3 days (green bars) fed with *Artemia* nauplii. (b) Feed intake (g egg custard/larval/day) of *M. rosenbergii* larvae exposed to neutral pH for 23 days (blue bars) and mild-acidic pH for 30 days (red bars) fed with egg custard. Data are represented as mean \pm SME with significantly levels indication as single asterisk (*) indicates $P < 0.05$; double asterisk (**) indicates $P < 0.01$ and triple asterisk (***) $P < 0.001$.

M. rosenbergii will then return to estuaries to spawn in the brackish water environment (Zafar et al., 2015). A good water quality, especially water pH plays a significant role in the early stage of larvae performance in ensuring the completion of such complex life cycle. Previous research had shown that *M. rosenbergii* juvenile is sensitive to water pH changing (Kawamura et al., 2015).

Environmental acidification occurring in nature via natural geographical composition is known to reduce water pH to an acidity level

in natural water bodies that is not suitable for most of the aquatic lives (Idrus et al., 2021). Nevertheless, environmental acidification caused by human-related activities via industrialization and agricultural pollution are known to cause spontaneous pH fluctuation (Thalib et al., 2021) and poses serious threats to aquatic ecosystems (Mohamad et al., 2021; Oliveira and de Santos, 2021). Research focus on the impact of freshwater acidification remains significantly limited as compared to ocean acidification (Hasler et al., 2017). Freshwater body is considered as acidic when

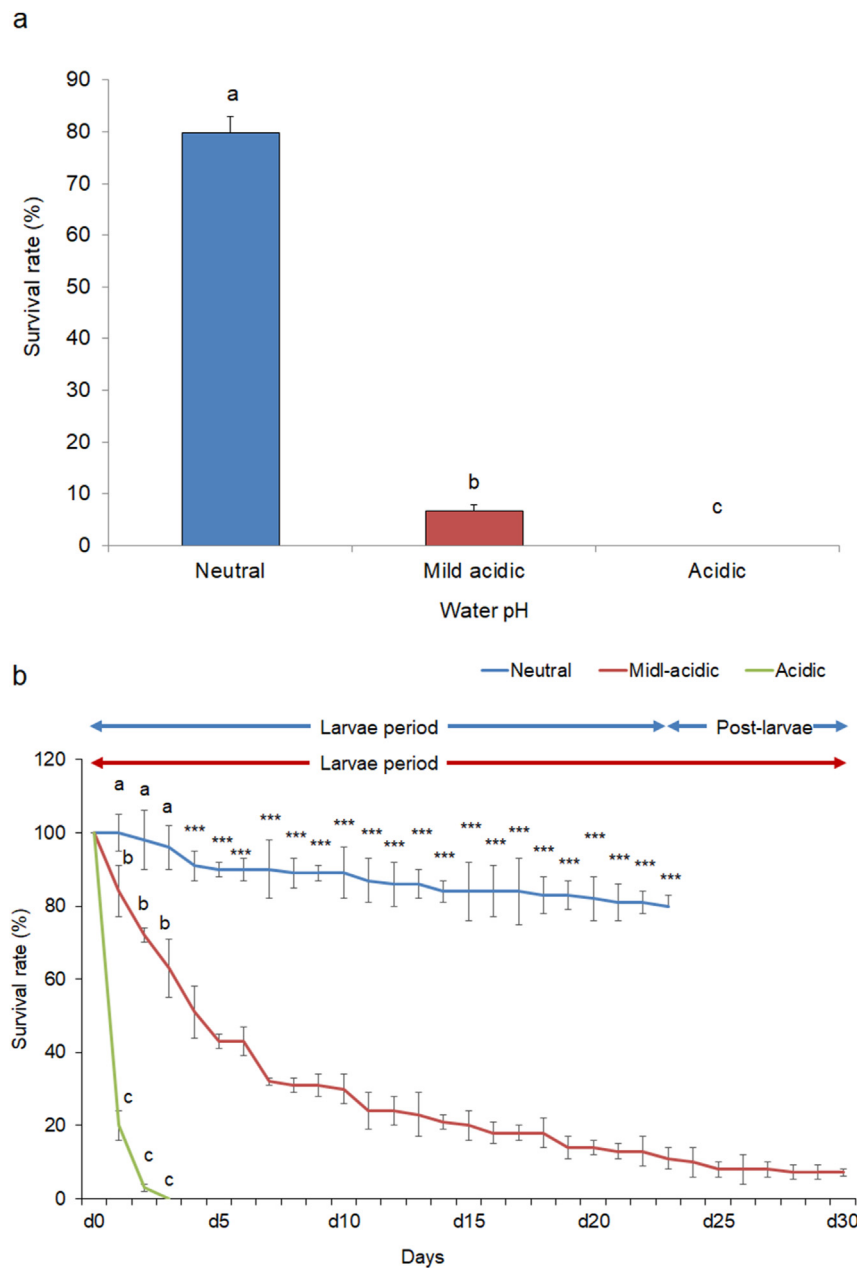


Figure 2. (a) Survival rate (%) of *M. rosenbergii* larvae exposed to neutral pH for 23 days (blue bar), mild-acidic pH for 30 days (red bar) and acidic pH for 3 days. (b) Daily survival rate (%) of *M. rosenbergii* larvae exposed to neutral pH for 23 days (blue line), mild-acidic pH for 30 days (red line) and acidic pH for 3 days (green line). Data are represented as mean ± SME with significantly levels indication as single asterisk (*) indicates $P < 0.05$; double asterisk (**) indicates $P < 0.01$ and triple asterisk (***) $P < 0.001$.

pH level drops below 6.5 due to the increasing emission of carbon dioxide (CO₂) and hydrogen ion (H⁺) into the water mainly caused by anthropogenic factors (Caldeira and Wickett, 2003; Beaune et al., 2018). Acidification is known to deteriorate environmental quality and distract natural food chain availability, thus depressing flora and fauna diversity (Freedman, 2018). Inland waters acidification could be a novel stressor to most of freshwater lives, where they may have no experience to confront with acidification scenario (Mohamad et al., 2021).

In nature, aquatic animals will migrate and swim to better living conditions to avoid unfavorable environments (Harkiolakis, 2013). In captivity, aquaculture species living in confined environment without migration opportunity will suffer from water pH fluctuation caused by terrestrial run-off or eutrophication issue. These conditions negatively impact the health status and/or more seriously result in mass mortality (Breeze, 2017), as evident in giant freshwater prawn farms (Kawamura et al., 2015; Masse et al., 2017; Amaral et al., 2020). The effects of acidification have been previously reported in post-larva (PL), juvenile, young and adult stages of *M. rosenbergii* (Chen and Chen, 2003;

Kawamura et al., 2015, 2018). However, to our knowledge, the effect of acidification on larval *M. rosenbergii* remains unclear, most probably due to the handling difficulty during the delicate larval stage. Therefore, this study was designed to examine the acute acidic pH impact on feeding, growth and early larval developmental performances of *M. rosenbergii*. As the production of *M. rosenbergii* is critically dependent on its early life stage performance, it is important to gain knowledge on the critical impact of the acidic pH towards early life performances of *M. rosenbergii*. With all this information as background, the objective of this study was to investigate the acute acidic pH impact on feeding, survival, growth and development performances of larvae *M. rosenbergii*.

2. Materials and methods

2.1. Source of specimen and management

Berried female giant freshwater prawns, *M. rosenbergii* were purchased from a commercial supplier in Manir, Kuala Terengganu and

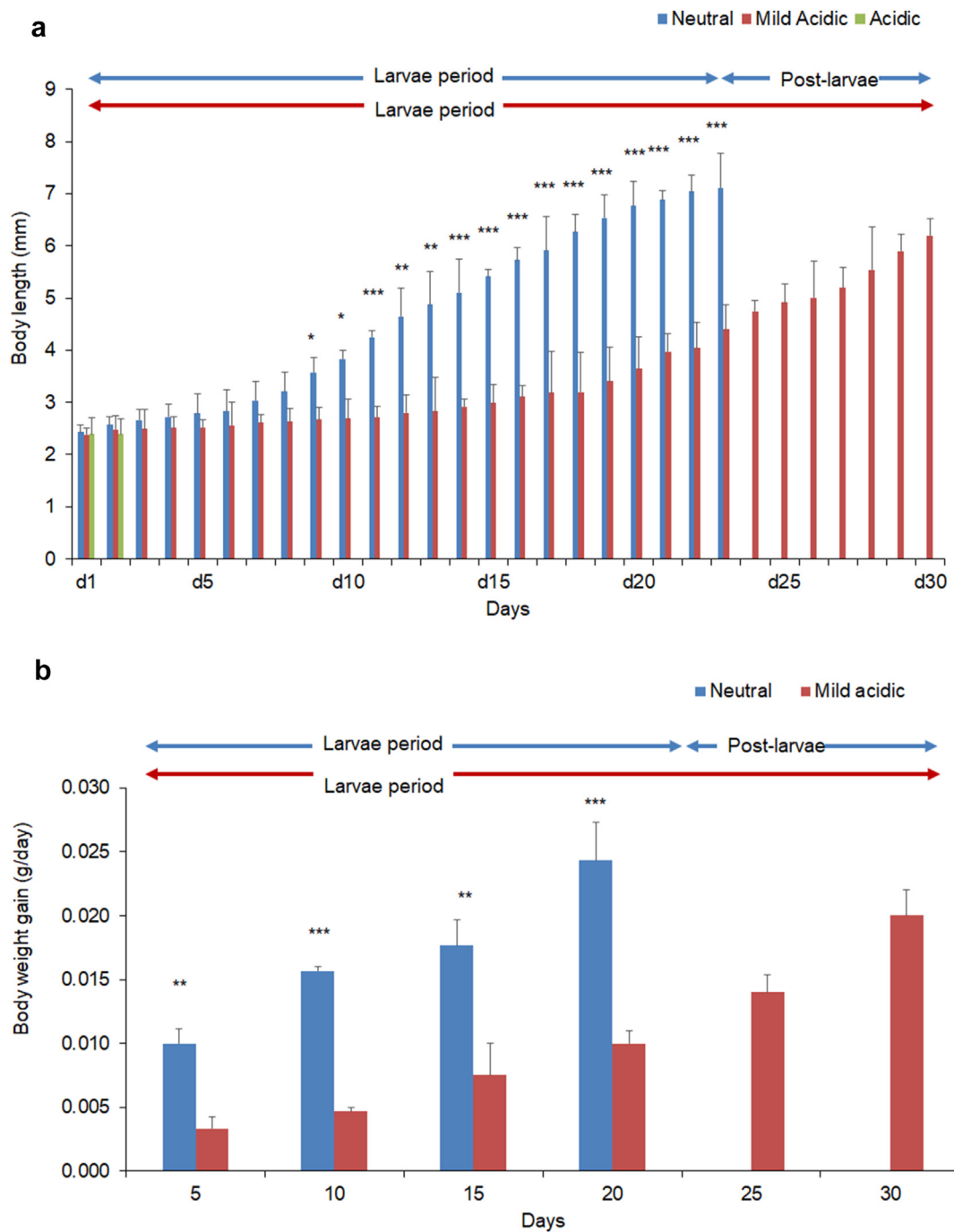


Figure 3. (a) Body length (mm) increment of *M. rosenbergii* larvae exposed to neutral pH for 23 days (blue bars), mild-acidic pH for 30 days (red bars) and acidic pH for 3 days (green bars). (b) Body weight increment (g/day) of *M. rosenbergii* larvae exposed to neutral pH for 23 days (blue bars) and mild-acidic pH for 30 days (red bars). Data are represented as mean \pm SME with significantly levels indication as single asterisk (*) indicates $P < 0.05$; double asterisk (**) indicates $P < 0.01$ and triple asterisk (***) $P < 0.001$.

transferred back to the hatchery facility at the Institute of Tropical Aquaculture and Fisheries, Universiti Malaysia Terengganu. A total of 10 berried females with the size of 68.85 ± 20.45 g for body weight (BW) and 12.23 ± 2.68 cm for body length (BL) were kept individually in a 50L conditional chamber equipped with a recirculation system at a salinity of 12 ppt for a week to examine egg maturation stage based on Muda et al. (2021). Feeding for all broodstocks were conducted twice a day at 8:00h and 18:00 h with squid cut into a size of approximately 1 cm^2 and fresh cockles at 3% BW. Uneaten food was removed after 1 h to avoid deterioration of water quality and 80% of the water was refreshed every two days. Water temperature, dissolved

oxygen, pH, salinity and ammonia were maintained at 28.5 ± 0.68 °C, 5.67 ± 0.48 mg/L, 7.8 ± 0.6 , 12.5 ± 0.6 ppt and 0.035 mg/L, respectively. When the eggs turned to greenish-grey colour and two black dots of eye formation were visible (Muda et al., 2021), the berried female was transferred to another 50 L rounded hatching tank with well-aerated 12 ppt water, and feeding was terminated until hatching occurred. After hatching, all newly hatched larvae (estimated at about 9000 larvae) were transferred to the larval tank for experimentation. The experiment was performed by using larvae produced from the same female. Thereafter, females were transferred back to the conditional chamber for optimisation.

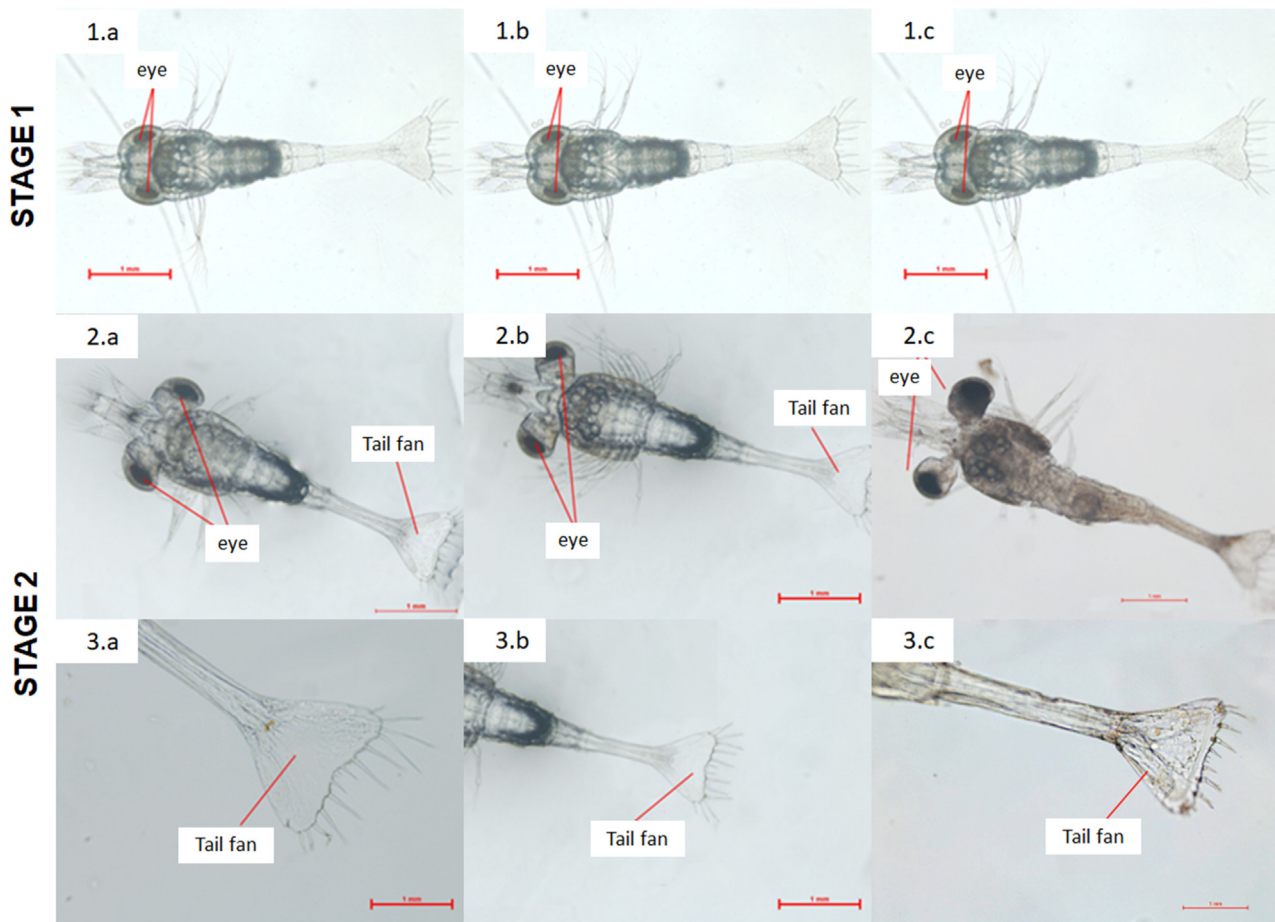


Figure 4. 1–3. Stage 1 and 2 developmental stages of *M. rosenbergii* larvae exposed to (a) neutral (pH 7.7 ± 0.4), (b) mild-acidic (pH 6.4 ± 0.5) and (c) acidic (pH 5.4 ± 0.2) conditions.

2.2. Experimental design

A single factorial experiment was constructed with water pH as a factor which were (i) neutral water pH at 7.67 ± 0.01 ; (ii) mild acidic water pH at 6.42 ± 0.36 ; and (iii) acidic water pH at 5.35 ± 0.01 in triplication. Acidic water pH was reduced using 1 mM hydrochloric acid (HCl) controlled by a peristaltic pump (SOGO, GB37-530) and the pH value was monitored every 3 h. Newly hatched larvae were randomly sampled and transferred into a semi-spherical fiberglass larval tank at a capacity of 500 L filled with well-aerated water at salinity of 12 ppt. A total of 9000 larvae were distributed randomly into 9 larval tanks at a stocking density of 1000 larvae per tank (2 larvae/L). Each experimental larval tank was equipped with 5 aeration stones located at corners and centre of the tank to create a complete water current circulation in the rearing tanks. This is important to allow a continuous movement of both food and larvae in a circular motion following the flow of the water current in the culture tank and to avoid the settlement issue due to low current. Feeding was given 3 times a day at 08:00 h, 14:00 h and 20:00 h with *Artemia* nauplii at a density of 60 nauplii/L. An additional *Artemia* nauplii at 50 nauplii/L were given every 2 days to ensure sufficient feed was given to support all larvae. At 10-days old, egg custard was introduced to the larvae and feeding frequency were increased to 5 times a day at 08:00 h, 11:00 h, 14:00 h, 17:00 h and 20:00 h together with nauplii *Artemia*. In order to provide an equal size of food, egg custard was scraped over a fine-mesh food sieve with a mesh size of 0.8 mm prior to feeding. Uneaten egg custard was siphoned after 1 h feeding. Daily 30% of water was replaced with fresh oxygenate water according to respective pH treatments (Table S1A–S1C). The experiment was terminated when the larvae metamorphose into the post-larval (PL) stage.

2.3. Egg custard preparation

The egg custard was prepared by using 10 g of high calcium milk powder, 1 egg, 0.2 g of fish oil, 0.01 g of complex vitamin, 0.01 g of vitamin C, 0.1 g of soy lecithin and 1 drop of food colouring (red colour to enhance attraction) (Abidin et al., 2010; Muda et al., 2021). All ingredients were mixed well and steamed for 15 min. The egg custard was left to cool at room temperature, cut into $2 \times 2 \text{ cm}^2$ and kept in a freezer. To ensure freshness quality, egg custard was prepared weekly.

2.4. Feeding and growth performances

Feeding and growth performances of *M. rosenbergii* were monitored throughout the experimental period. In order to monitor the feeding rate of *Artemia* nauplii, food density at initial and 1 h post feeding was estimated by collecting 100 ml water sample from each tank in 5 replications. Number of *Artemia* nauplii were counted using the Sedgwick-Rafter cells counting slide and the feed intake was expressed as the number of *Artemia* per individual larvae daily (*Artemia*/larvae/day). At the same time, the number of *M. rosenbergii* larvae was counted to determine the survival rate. On the other hand, the egg custard intake volume was expressed as the amount of egg custard consumed per larval per day (g egg custard/larvae/day). Uneaten egg custard was siphoned-out, blotted dry and weighed. Body length of the larvae were measured from the anterior-most part of the larvae to the end of the tail. The images of body development were taken by using the Measuring Profile Projector (Nikon MM – 800, Japan). Body length and weight measurements were performed daily in each culture tank by randomly sampling 10 larvae in 5 replications. Measurements were monitored daily until the larvae

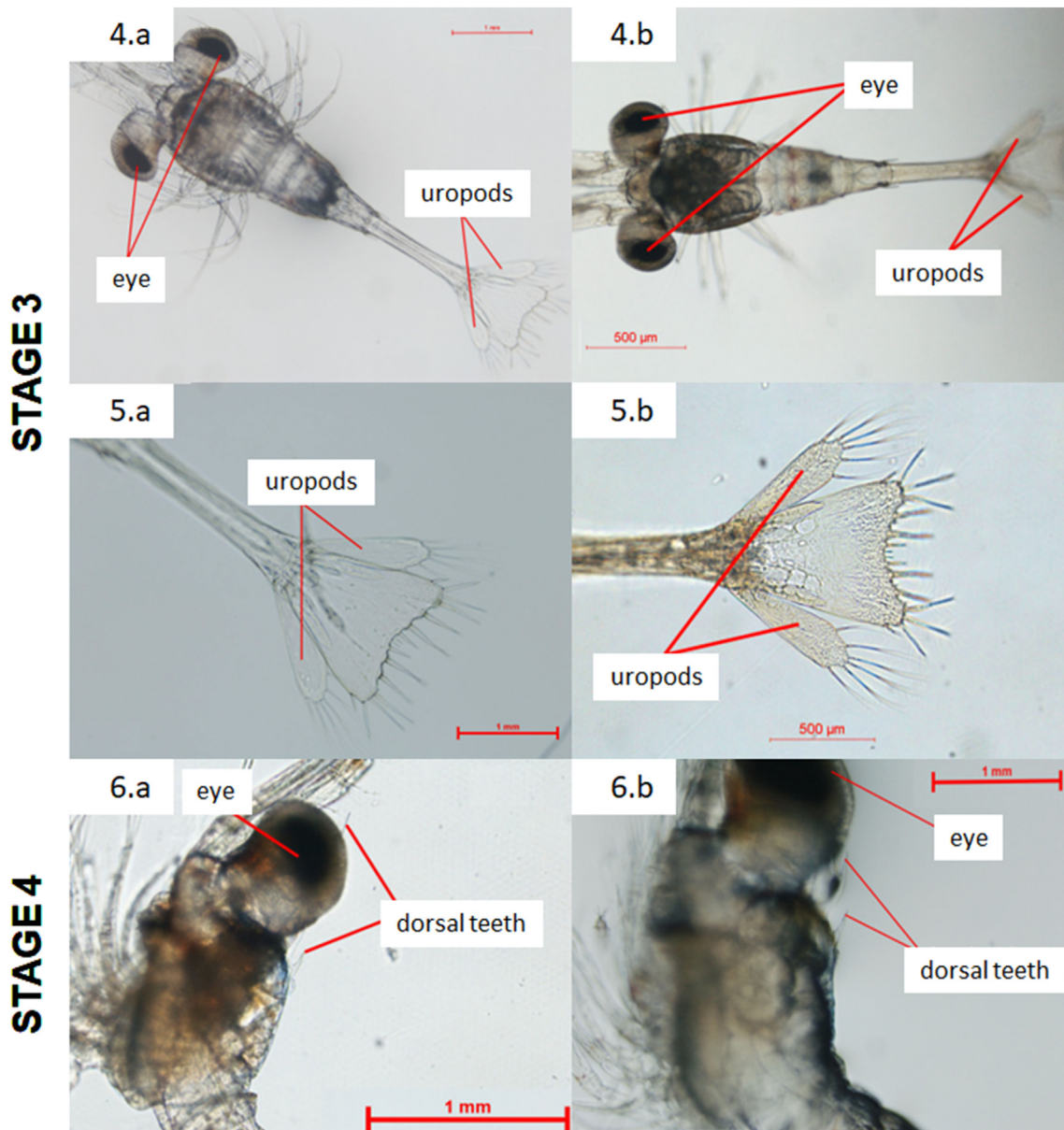


Figure 4. 4–6. Stage 3 and 4 developmental stages of *M. rosenbergii* larvae exposed to (a) neutral (pH 7.7 ± 0.4) and (b) mild-acidic (pH 6.4 ± 0.5) conditions.

successfully metamorphosed into the post-larvae stage. For body weight, a total of 10 larvae were collected and measured using a digital balance up to 4 decimal points (Mettler Toledo, Switzerland). As the body weight increment was relatively small, therefore the body weight measurement was performed every 5-days intervals. Survival, weight gain, body length increment and feed intake were calculated as follows:

- i. Feed intake (*Artemia*/larvae/day) = ((initial number of *Artemia* – final number of *Artemia*) × total water volume)/total number of larvae/day
- ii. Feed intake (g egg custard/larval/day) = (initial volume egg custard – final volume egg custard × total water volume)/total number of larvae/day
- iii. Survival rate (%) = (initial number of larvae – number of dead larvae)/initial number of larvae × 100
- iv. Length increment (mm) = total body length measurement taken from the anterior-most part of the larvae to the end of the tail
- v. Weight gain (g/day) = (final body weight – initial body weight)/time

2.5. Statistical analysis

The feeding and growth performances of *M. rosenbergii* larvae were compared under three different water pH (neutral, mild acidic and acidic conditions). All data collected were analysed by using one-way analysis of variance (ANOVA) followed by Tukey post-hoc test. A probability level of 0.05 was used to reject the null hypothesis. Prior to perform ANOVA, all data were checked for normality distribution by using Shapiro-Wilk. Data that did not fulfil the ANOVA requirement, log-transformed data were used for analysis. Non-parametric analysis was performed by using Kruskal-Wallis, if the transformed data still fail to meet the normality distribution. Nevertheless, as the *M. rosenbergii* larvae were not able to survive beyond 48h exposure to acidic pH, feeding and growth performances were assessed between neutral pH and mild-acidic pH by using the unpaired two-tail student *t*-test. Significance level was set at least 95% ($P < 0.05$), 99% ($P < 0.01$) or 99.9% ($P < 0.001$) confident limit. All the data were analysed by using the Statistical Package for the Social Sciences (SPSS) software version 20.0. All the data are presented as mean values ± SME (standard mean error).

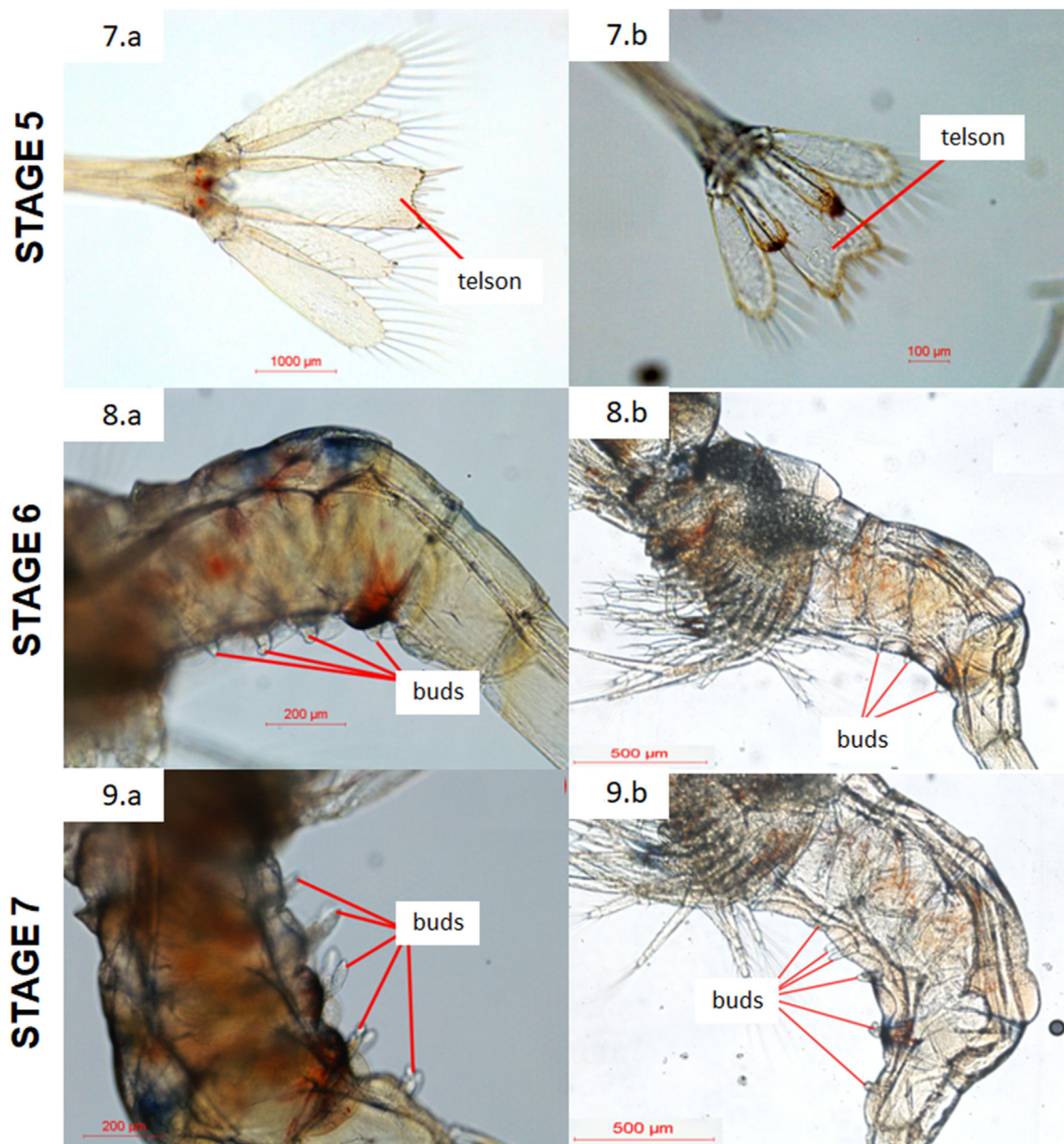


Figure 4. 7–9. Stage 5, 6 and 7 developmental stages of *M. rosenbergii* larvae exposed to (a) neutral (pH 7.7 ± 0.4) and (b) mild-acidic (pH 6.4 ± 0.5) conditions.

3. Results

3.1. Feeding performance of *M. rosenbergii* larvae

As expected, *M. rosenbergii* larvae cultured under neutral pH consumed the highest number of *Artemia* from the beginning of the experiment until day-17 (198 ± 9.02 *Artemia*/larvae/day) before decreasing to 151 ± 6.04 *Artemia*/larvae/day until day-23 (Figure 1a). Similarly, under mild-acidic condition, the *Artemia* consumption rate increased as the larvae grew and the highest *Artemia* consumption rate was recorded on day-21 with 170 ± 6.55 *Artemia*/larvae/day. Comparatively, however, the *Artemia* consumption rate was significantly lower in larvae cultured under mild-acidic conditions compared to those cultured under neutral pH ($P < 0.05$; Figure 1a; Table S2).

Egg custard was given on day-10 to both neutral and mild-acidic pH larvae to optimize their feeding efficiency. Similar to *Artemia*, slower increment of feeding pattern and lower average consumption rate in larvae subjected to mild-acidic pH were observed when fed with egg custard. Under neutral pH, feeding was at its peak on day-23 (4.86 ± 0.26

g egg custard/larval/day), whereas peak was a week later on day-30 (2.84 ± 0.25 g egg custard/larval/day) for the larvae cultured under mild-acidic condition ($P < 0.05$; Figure 1b; Table S3).

3.2. Survival rate of *M. rosenbergii* larvae

Only larvae cultured under neutral and mild-acidic pH water were able to survive and successfully metamorphosed into the post-larval (PL) stage. However, larvae cultured under neutral pH condition achieved 80% survival rate compared to only 7% survival rate was recorded in larvae cultured under mild-acidic pH condition. None of the larvae cultured in acidic pH water condition survived more than 48 h ($P < 0.001$; Figure 2a and b). Although under neutral pH condition, death of *M. rosenbergii* larvae was noticed on day-10 and the number of surviving larvae started to decrease gradually, larvae started to metamorphose to post-larval (PL) stage on day-22 (Figure 1b). Comparatively, *M. rosenbergii* larvae that were cultured under mild-acidic condition started to die on day-2 and continuously decreased till day-28 before metamorphosing into the PL stage on day-30 (Figure 1b; Table S4).

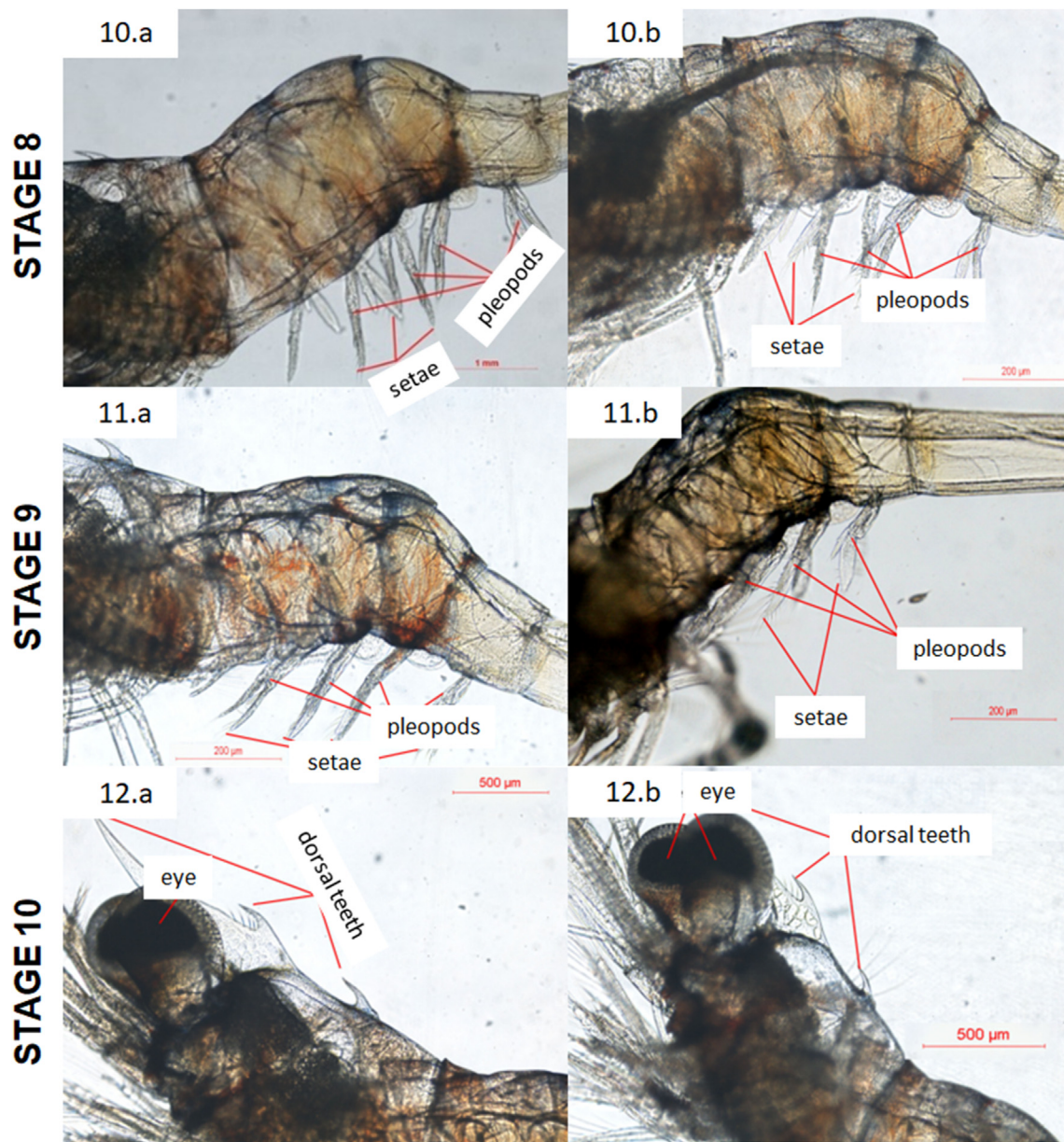


Figure 4. 10–12. Stage 8, 9 and 10 developmental stages of *M. rosenbergii* larvae exposed to (a) neutral (pH 7.7 ± 0.4) and (b) mild-acidic (pH 6.4 ± 0.5) conditions.

3.3. Growth performances of *M. rosenbergii* larvae

Based on the daily comparison, a significant difference in length increment was visible from day-9 onwards up to day-23, with larvae cultured under neutral pH showed longer body length (3.47 ± 0.35 mm) compared to mild-acidic pH conditions (2.62 ± 0.26 mm) ($P < 0.05$, Figure 3a). Under neutral pH condition, the body length of newly metamorphosed post-larvae was 6.82 ± 0.58 mm on day-23. Comparatively, the newly metamorphosed post-larvae on day-30 under the mild-acidic pH treatment was only 5.92 ± 0.73 mm ($P < 0.05$; Figure 3a; Table S5).

Body weight gain measurement was performed every 5 days until the larvae metamorphosed into PL (Figure 3b). Due to the 100% mortality rate of larvae subjected to acidic pH treatment, data were only analysed for larvae cultured under neutral and mild-acidic pH. Results clearly showed that larvae cultured under neutral pH condition exhibited about 1.5-fold heavier weight gain compared to larvae cultured under mild-acidic condition ($P < 0.05$, Figure 3b). The greatest weight gain of larvae cultured under neutral pH was recorded at 0.0245 ± 0.0048

larvae/g/day on day-23 prior to metamorphosis into PL, whereas, under mild-acidic condition, the highest body weight gain was noted at 0.0201 ± 0.0031 larvae/g/day on day-30 (Figure 3b; Table S6).

3.4. Early developmental of *M. rosenbergii* larvae

The *M. rosenbergii* larval development, as characterized by their morphometric changes under neutral, mild-acidic and acidic pH was illustrated in Figure 4 and described in Table 1. Based on the morphological observations, water pH influenced each larval development stage (Figure 4.1a-c). At stage-2, physical deformation, such as asymmetrical eyestalks were already obvious in larvae under acidic pH treatment (Figure 4.2c) compared to the symmetrical eyestalks in mild-acidic and neutral pH (Figure 4.2a & 4.2.b). Other deformity experienced by larvae under acidic pH treatment includes deformed tail fan (Figure 4.3c), but not for neutral and mild-acidic pH treatments (Figure 4.3a-b). As a result of their inferior physical appearance in poor condition, the larvae under acidic treatment were not able to survive to the next stage, therefore no observation was recorded.

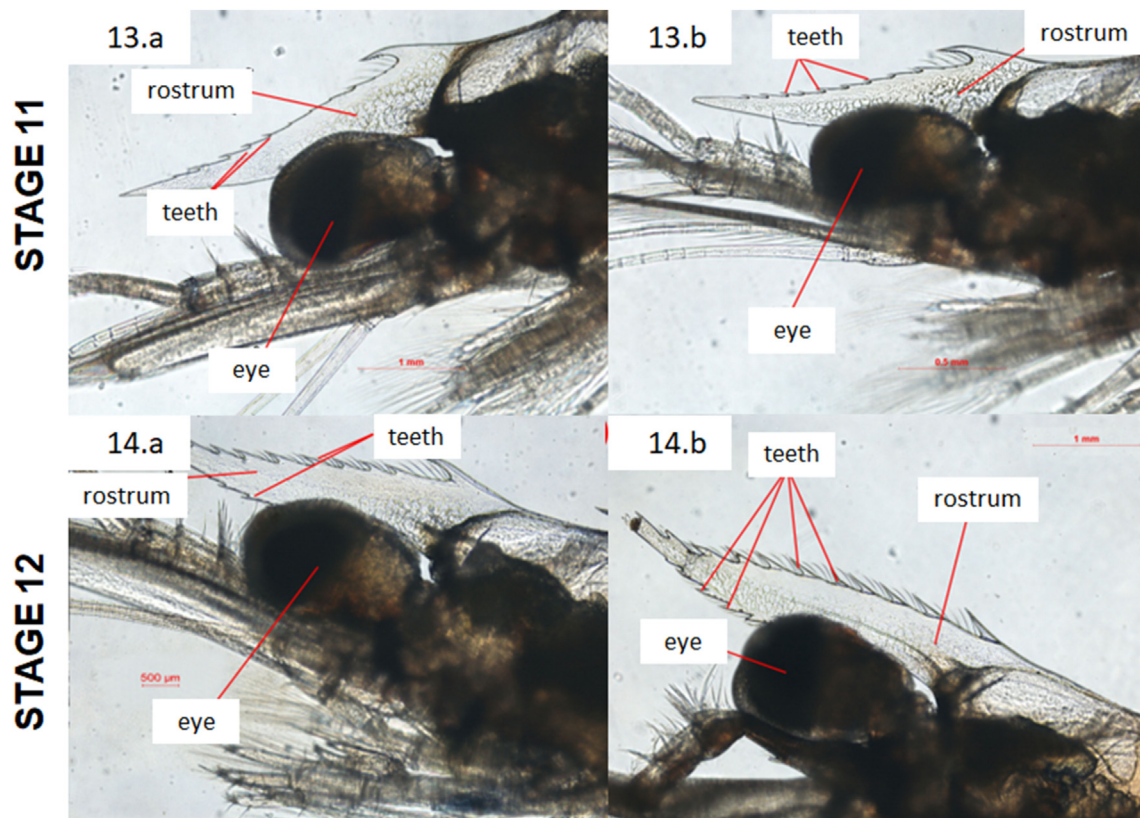


Figure 4. 13–14. Stage 11 and 12 developmental stages of *M. rosenbergii* larvae exposed to (a) neutral ($\text{pH } 7.7 \pm 0.4$) and (b) mild-acidic ($\text{pH } 6.4 \pm 0.5$) conditions.

In the stage 3, the uropods of larvae under mild-acidic conditions turned to a slightly brownish colour compared to the clear transparent shape observed in larvae under neutral condition (Figure 4.4a-b. & 4.5.a-b). The distinctive formation of dorsal rostrum teeth in stage 4 and the development of new character on their telson were observed in both water pH conditions (Figure 4.6a-b). However, the telson became narrow and elongated under neutral pH with 4 equal size uropods (Figure 4.7.a). In comparison to the larvae under mild-acidic condition, the telson was with less narrow elongated and unequal uropod development with only two well-developed uropods (Figure 4.7.b).

Under neutral condition, larvae achieved stage 6 on day-7 and exhibited developed pleopod buds (Figure 4.8.a), whilst the pleopod buds of stage-6 larvae in mild-acidic pH treatment (on day-11) were small and under-developed (Figure 4.8.b). Entering stage 7, the pleopods buds of larvae under neutral pH condition became biramous (Figure 4.9.a), but not for the larvae under mild-acidic condition (Figure 4.9.b). As the larvae continue to develop to stage 8, there were not many differences between the two pH treatments, except that setae were obvious on the pleopod buds of larvae under neutral pH treatment (Figure 4.10.a), whilst larvae exposed to mild-acidic pH was barely visible (Figure 4.10.b).

In stage 9, pleopods and endopods with long setae were only obvious for larvae under neutral pH treatment (Figure 4.11.a and 11.b). As observed under mild-acidic pH treatment, the pleopod buds of larvae were slightly shortened (Figure 4.11.b). At stage 10, the appearance of three dorsal teeth on the rostrum was noticed in both pH treatments (Figure 4.11.a and b). However, larvae that were exposed to mild-acidic pH exhibited slightly shortened rostrum compared to those exposed to neutral pH (Figure 4.12.b).

At the final larval stage (stage 11) prior to metamorphosis into post-larvae, obvious difference in the rostrum character teeth on the half upper dorsal margin of the rostrum was observed between pH treatments.

Sharp rostrum tip appeared on the larvae under neutral pH (Figure 4.13.a), while dull with short rostrum teeth characteristics were noticed on the larvae reared under mild-acidic pH (Figure 4.13.b).

After completing the larval stages, the larvae metamorphosed into the PL stage, with significant physical and behavioural changes (Figure 4.14). PL started to settle at the bottom with an active mobile crawl searching for food and able to move and swim forward. Newly metamorphosed PL developed teeth on the dorsal and ventral part of their rostrum (Figure 4.14). However, under mild-acidic condition, deformities (broken and dull tip) occurred at the tip of the rostrum part (Figure 4.14.a) compared to the tip of the rostrum part in larvae under neutral pH which appeared healthy and sharp (Figure 4.14.b).

4. Discussion

4.1. Effect of low pH on feeding and growth performances of *M. rosenbergii* larvae

Water pH is one of the most critical abiotic factors affecting aquatic life's performance from physical actions to physiological cellular responses. Sensitivity response towards water pH fluctuation depends on species-specific tolerances, life stages and exposure levels, whether in wild or captive conditions (Chen and Chen, 2003; Kawamura et al., 2015; Mohamad et al., 2021; Thalib et al., 2021). In freshwater medium, environment pH ranging from 6.0 to 8.5 is considered as natural pH for aquatic life (Tucker and D'Abramo, 2008). Nevertheless, the pH ranging between 7.5 – 8.5 was reported to be the optimum pH for growth promotion of *M. rosenbergii* (New, 2005), where the haemolymph pH is maintained at 7.6 (Yeh et al., 2006). Although our study focused on early larvae stage, our result was in agreement with New (2005), where the highest survival rate and growth performances were found in *M. rosenbergii* larvae under neutral pH (7.67 ± 0.01) compared to

Table 1. Developmental stages of *M. rosenbergii* larvae exposed to neutral (pH 7.7 ± 0.4), mild-acidic (pH 6.4 ± 0.5) and acidic (pH 5.4 ± 0.2) conditions.

| Stages | Neutral | Mild acidic | Acidic |
|------------------------|--|---|--|
| Stage 1 | Sessile eyes | Sessile eyes | Sessile eyes |
| Stage 2 | <ul style="list-style-type: none"> Symmetrical eyes stalk Healthy body shape | <ul style="list-style-type: none"> Symmetrical eyes stalk Healthy body shape | <ul style="list-style-type: none"> Asymmetrical eyes stalk Deformed body shape |
| Stage 3 | Uropods appear | Cloudy uropods appear | N/A |
| Stage 4 | 2 dorsal teeth on rostrum appear | 2 dorsal teeth on rostrum appear | N/A |
| Stage 5 | Telson become narrower and elongated | Telson become slightly narrower and elongated | N/A |
| Stage 6 | Pleopods buds appear | Pleopods buds barely appear | N/A |
| Stage 7 | Biramous pleopods | Barely biramous pleopods | N/A |
| Stage 8 | Pleopods with setae | <ul style="list-style-type: none"> Barely setae of pleopods Length of pleopods slightly short | N/A |
| Stage 9 | Pleopods and endopods with longer setae | <ul style="list-style-type: none"> Barely longer setae of pleopods Length of pleopods slightly short | N/A |
| Stage 10 | 3 dorsal teeth appear on rostrum | <ul style="list-style-type: none"> 3 dorsal teeth appear on rostrum Total length of rostrum slightly short | N/A |
| Stage 11 | <ul style="list-style-type: none"> Teeth on half upper dorsal margin of rostrum appear Tip of rostrum appear sharp | <ul style="list-style-type: none"> Teeth on upper and lower part of rostrum appear Tip of rostrum appear dull | N/A |
| Stage 12 (Post-larvae) | <ul style="list-style-type: none"> Teeth on upper and lower part of rostrum appear Tip of rostrum appear sharp | <ul style="list-style-type: none"> Teeth on upper and lower part of rostrum appear Tip of rostrum appear deform | N/A |

N/A indicates data not available, where larvae survive under acidic pH exposure.

mild-acidic pH (6.42 ± 0.36). Our results also proved that *M. rosenbergii* larvae are highly sensitive to acidic pH, where no larvae were able to survive beyond 48 h under acidic pH (5.35 ± 0.01).

Apart from the low survival rate, low water pH also significantly suppressed feed intake of *M. rosenbergii* larvae, as evident in the reduced consumption rate of *Artemia* or egg custard. Feeding in crustacean species depends on chemosensory and mechanoreceptor responses using arthropod cuticles and setae to detect food items (Wainwright et al., 1976). Under low pH exposure, *M. rosenbergii* PL and juvenile challenged at pH 4 decalcified setae and impacted its feeding function (Kawamura et al., 2015). Similar reduced feeding efficiency due to reduced pH was observed in the present study, which consequently it led to low body weight gain and body length increment as shown in Figure 3. This highlighted the sensitivity of *M. rosenbergii* during the early life stage to acidic water pH. In order to maintain high survival production, *M. rosenbergii* larvae should be maintained at water pH 7–8.

Exposure to acidic pH adversely suppressed feed intake (Abbink et al., 2012; Kennedy and Picard, 2012), and resulted in lower growth and survival of crustacean larvae (Tucker and D'Abamo, 2008; Kennedy and Picard, 2012; Almut and Bamber, 2013; Giltz and Taylor, 2017). In previous studies, low water pH depressed foraging sensitivity and feeding performances in PL and juveniles of *M. rosenbergii* (Kawamura et al., 2018). Similar impairment of feeding was also observed in other crustacean species (Kennedy and Picard, 2012) such as tiger prawn *Penaeus monodon* (Chen and Lin, 1992), Northern shrimp *Pandalus borealis* (Arnberg et al., 2013), red rock shrimp *Lysmata californica* (Taylor et al., 2015), Pacific white shrimp *Litopenaeus vannamei* (Yu et al., 2020), swimming crab *Portunus trituberculatus* (Lin et al., 2020), kuruma shrimp *Marsupenaeus japonicus* (Chen et al., 1996; Fukami et al., 2021), and teleost such as cobia *Rachycentron canadum* (Rodrigues et al., 2013), African catfish *Clarias gariepinus* and tilapia *Oreochromis niloticus* (Mota et al., 2015), turbot *Psetta maxima* (Mota et al., 2018) and hybrid grouper *Epinephelus lanceolatus* × *Epinephelus fuscoguttatus* (Thalib et al., 2021).

4.2. Effect of low pH on early development of *M. rosenbergii* larvae

In addition to feeding, larval development of *M. rosenbergii* larvae was also significantly impaired by acidic water pH, with prolonged exposure to mild-acidic pH resulted in malformation of morphological characters and extended larvae stages before metamorphosing into the PL stage (Figure 4). The adverse effect of acidic pH on morphological development (abnormal eye and uropod formation) and pigmentation (yellow

discolouration) were noticeable as early as stage 2 (Figure 4.2). Delayed development in *M. rosenbergii* larvae became obvious as larvae approached stage 5 and beyond. Physiologically, the poor performances of *M. rosenbergii* larvae under acidic pH were due to osmotic disturbance that affects the osmolality and ionoregulation to maintain effective basal metabolism for optimum growth and development. An impairment of osmoregulation under acidic pH not only inhibits essential ion uptake, but also increases the passive ion efflux (Allan and Maguire, 1992). Previous studies revealed a net loss of Cl⁻ and Na⁺ when organisms were subjected to acidic pH (Zanotto and Wheatly, 1993) as Cl⁻ influx and HCO₃⁻ efflux were used to regulate hemolymph pH and acid-base balance via Cl⁻/HCO₃⁻ exchanger. Meanwhile, Na⁺ influx and H⁺ efflux via Na⁺/H⁺ exchanger was used to regulate Na⁺ transportation in crayfish when exposed to low water pH (Henry and Wheatly, 1992; Chen and Chen, 2003). The poor morphological development of *M. rosenbergii* larvae exposed to acidic pH observed in this study could also be attributed to the disturbance in the ionoregulation mechanism. Similar poor feeding efficiency and prolonged larvae period with low survival and growth were also reported in other crustacean species (Taylor et al., 2015; Lin et al., 2020). Furthermore, the higher 96-h LC50 of pH of *M. rosenbergii* compared to other crustacean species highlights the higher sensitivity of this species towards acidic pH (Chen and Chen, 2003). The negative impacts of acidification on early life development were also documented in other shellfish species including oyster *Saccostrea glomerata* (Wilson and Hyne, 1997), clam *Mercenaria mercenaria* (Kurihara, 2008), sea urchin *Paracentrotus lividus* (Byrne et al., 2009), scallop *Placopecten magellanicus* (Desrosiers et al., 1996), and sea star *Crossaster papposus* (Dupont et al., 2010). Acidic pH suppresses development by affecting (downregulating) biomineralisation and skeletogenesis, consequently resulted in morphological abnormality and/or mass mortality during the sensitive early life stages of shellfish species (O'Donnell et al., 2009; Ross et al., 2011).

5. Conclusion

In conclusion, this study summarised that *M. rosenbergii* larvae are highly sensitive to acidic water pH. To secure mass production and optimal *M. rosenbergii* larvae development with a high survival rate, water pH should be maintained at a neutral level of pH 7.5. Although *M. rosenbergii* larvae were able to tolerate mild-acidic pH 6.5, it resulted in prolonged larvae period and led to poor morphological development. Acidic pH of 5.35 and below is lethal to *M. rosenbergii* larvae, where no

larvae were able to withstand more than 48 h. Future investigation should focus on ion transporters and enzyme activity to uncover the ionoregulatory functionality of crustacean larvae under acidification scenario.

Declarations

Author contribution statement

Hon Jung Liew, Sharifah Rahmah & Mazlan Abd Ghaffar: Conceived and designed the experiments; Wrote the paper.

Pei Wen Tang & Siti Izzah Athirah Hamin: Performed the experiments.

Khor Waiho, Hanafiah Fazhan, Nadiah Wan Rasdi & Leong-Seng Lim: Analyzed and interpreted the data.

Suhairi Mazelan & Sabri Muda: Contributed research materials, analysis tools or data.

Young-Mou Chen: Conceived and designed the experiments.

Yu Mei Chang & Li Qun Liang - Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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