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Article

Low pH Affects Survival, Growth, Size Distribution, and Carapace Quality of the Postlarvae and Early Juveniles of the Freshwater Prawn *Macrobrachium rosenbergii* de Man

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Abstract – Acidification of rain water caused by air pollutants is now recognized as a serious threat to aquatic ecosystems. We examined the effects of low pH (control pH 7.5, pH 6, pH 5, pH 4) on the survival, growth, and shell quality of *Macrobrachium rosenbergii* postlarvae and early juveniles in the laboratory. Hatchery-produced postlarvae (PL 5) were stocked at 250 PL per aquarium, acclimated over 7 d to experimental pH adjusted with hydrochloric acid, and reared for 30 d. Dead specimens were removed and counted twice a day. After 27 d rearing, all specimens were measured for total length and body weight. Carapace quality was assessed by spectrophotometry. Survival of juveniles was highest at pH 6 (binomial 95% confidence interval 79 - 89%) followed by control pH 7.5 (56 - 68%) and pH 5 (50 - 60%) and was lowest for unmetamorphosed postlarvae and juveniles at pH 4 (43 - 49%). The final median total length and body weight of juveniles were similar at control pH 7.5 (18.2 TL, 50.2 mg BW) and pH 6 (17.7 mm TL, 45.0 mg BW) but significantly less at pH 5 (16.7 mm TL, 38.2 mg BW); at pH 4, the postlarvae did not metamorphose and measured only 9.8 mm TL, 29.3 mg BW. Length frequency distribution showed homogeneous growth at pH 6, positive skew at control pH 7.5 and pH 5, and extreme heterogeneity at pH 4. The carapace showed different transmittance spectra and lower total transmittance (i.e. thicker carapace) in juveniles at pH 7.5, pH 6, and pH 5 than in unmetamorphosed postlarvae and juveniles with thinner carapace at pH 4. Thus, survival, growth, size distribution, and carapace quality of *M. rosenbergii* postlarvae and early juveniles were negatively affected by pH 5 and especially pH 4. The thinner carapace of the survivors at pH 4 was mostly due to their small size and failure to metamorphose. Natural waters affected by acid rain could decimate *M. rosenbergii* populations in the wild.

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Key words – acidification, pH, *Macrobrachium rosenbergii,* size heterogeneity, carapace quality

1. Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*) inhabits rivers, ponds, lakes, and estuaries in south and southeast Asia, northern Oceania, and the western Pacific islands. Berried females migrate downstream to estuaries where the eggs are released and the larvae hatch as zoea; after metamorphosis, the postlarvae migrate into rivers and lakes (Ismael and New 2000). In natural freshwater ponds, pH levels normally fluctuate due to the removal of carbon dioxide by phytoplankton during the day and its release at night (Boyd and Zimmermann 2000). Problems with high pH are common in freshwater ponds used to grow *M. rosenbergii* because fertilizers applied during pond preparation promote phytoplankton blooms that rapidly take up carbon dioxide (Boyd and Zimmermann 2000). The negative effects of high pH on larvae and postlarvae in ponds and in the laboratory are well documented (Alston and Sampaio 2000).

On the other hand, aquaculture of *M. rosenbergii* is affected by low pond water pH (Chen and Chen 2003) due to the acidification of rain water, now recognized as a serious threat to aquatic ecosystems (Alston and Sampaio 2000). Air pollutants are emitted into the atmosphere from anthropogenic sources and travel across national boundaries. Water is a powerful solvent and natural waters are never pure. Normal pure rainwater usually has a pH of about 6.0 or above (Tucker and D'Abramo 2008). Acid rain contributes to the acidification of river and lake waters (Haines 1981). In remote areas such as the wildlife sanctuary in Miri, Sarawak, the river water ranged from pH 6.02 to pH 6.74 (Gandaseca et al. 2011). Elsewhere in Malaysia, acidic water in rivers and lakes has been reported: pH 3.2 - 6.3 in Tasik Chini's Feeder River in Pahang (Gasim et al. 2006); pH 4.5 - 6.8 in Chini Lake, Pahang (Shuhaimi-Othman et al. 2008); pH 3.2 - 6.7 at 17 sampling sites in Langat River flowing through oil palm and rubber plantations in Selangor (Juahir et al. 2009); pH 3.8 - 5.4 at Nilai Industrial Park, Negeri Sembilan (Norela et al. 2009); and pH 4.9 - 5.9 at Danum Valley, Sabah (Sumari et al. 2009). In the remote Danum Valley, about 17% of the total rainfall was acidic (pH < 5.0) and 75% was mildly acidic (pH 5.0 - 5.6) (Sumari et al. 2009). Aquaculture of *M. rosenbergii* in the Mekong Delta of Vietnam is greatly dependent on prawn juveniles collected from the wild (Wilder et al. 1999), and pH < 4 has been recorded at several hydrostations in the delta in 2008 (Anonymous 2011).

Knowledge on the effect of low pH on *M. rosenbergii* contributes to the understanding of the species ecology and to improvement of hatchery management. The present study examined the effects of acidic rearing water at pH 6, pH 5, and pH 4 (against the control at pH 7.5) on the survival, growth, length and weight distribution, and carapace shell quality of *M. rosenbergii* postlarvae and early juveniles in the laboratory.

2. Materials and Methods

Source of experimental animals

Zoeae of the freshwater giant prawn were reared to postlarvae PL5 at water temperature 25.7 - 27.4°C; salinity 8 - 12 ppt; and pH 7.4 - 8.9 at the Shrimp Hatchery of the Borneo Marine Research Institute, Universiti Malaysia Sabah. Larvae were fed with formulated diet (CP Aquaculture Limited, Thailand). The postlarvae were gradually acclimated to freshwater over 7 days in the shrimp hatchery before the pH experiment was conducted in the adjoining wet laboratory. Care and handling of experimental animals followed the guidelines set by the World Health Organization (WHO, Geneva, Switzerland); the Malaysian Animal Handling Code of Conduct; and the National Research Council (1996) guide for the care and use of laboratory animals.

pH experiment

Four glass aquaria (60 cm long × 30 cm wide × 30 cm high) were arranged at random in the laboratory and filled with freshwater to a depth of 25 cm. The aquaria were rearranged at random every 2 d (by means of the table of random numbers) to expose the postlarvae in the four tanks to all possible ambient conditions and avoid nuisance factors. Each aquarium had a cubic three-layer plastic netting substrate and an air-lift water filtration unit. One aquarium was maintained at the ambient water pH 7.5 (control) and the three aquaria were adjusted to water pH 6, pH 5, and pH 4 by adding hydrochloric acid at 11.5 mM HCl such that pH 7.5 was reduced at the rate of 1 pH unit/day. For the pH 4 aquarium, slower addition of HCl was necessary after reaching pH 5 to reduce pH at the rate of 0.2 pH unit/day until pH 4; a change from pH 5 to pH 4 in one day caused total mortality in a preliminary study. Adjustment from pH 7.5 to pH 4 took 7 days. Adjustment of experimental pH by addition of HCl is a commonly used method (Pan et al. 2005; Ravi & Manisseri 2013). The aquarium water was replaced every day, 10% of volume in the morning and 90% in the afternoon, with new water of the respective pH (pH-adjusted before addition). About 30 min after water replacement, pH, temperature, salinity, and dissolved oxygen in the aquaria were measured with a pH/ORD/EC/DO tester (Hanna Instruments, HI 9828). Table 1 shows the water quality in the experimental aquaria.

Postlarvae (initial stage PL5) were stocked at 250 per experimental aquarium and reared in the pH treatments for 27 - 34 d, fed the same pelleted diet. Dead postlarvae were

Table 1. Water quality parameters in the test aquaria

pH treatment	pH		Salinity (ppt)	Temperature (ºC)	Dissolved oxygen (ppm)
	Range	Median	Range	Range	Range
Control pH 7.5	7.24–7.62	7.51	0.06–0.14	25.5–28.0	5.4–6.7
pH 6	5.85–6.21	6.03	0.07–0.08	25.7–28.1	5.9–6.8
pH 5	4.75–5.19	4.97	0.06–0.35	25.4–29.0	5.8–6.5
pH 4	4.04–4.28	4.13	0.07–0.10	25.4–28.7	5.7–7.0

removed from the aquaria and recorded twice a day. After the experiment, all live specimens were anaesthetized in ice water and measured for TL and wet body weight (BW). TL was measured to 0.01 mm with a digital caliper gauge (SHINWA RULES, Niigata, Japan) under a dissection light microscope (NIKON, SMZ645, Tokyo, Japan). BW was measured to 0.01 g with an electronic balance (Precisa 404A, Dietikon, Switzerland).

Spectrophotometry of the carapace shell

Prawn postlarvae and early juveniles are completely transparent (Ling 1962). In our observation, pigmentation of the carapace did not take place even in late juveniles. The carapace shell quality of specimens reared at low pH was assessed by spectrophotometry given that thinner shells transmit more light and low transmittance indicates thicker shells. After the TL and BW measurements, the carapace shells of 9 specimens were removed from pH 4 and 7 from the other treatments and washed in distilled water for spectrophotometry. Thinner shells transmit more light than thicker shells, and low transmittance indicates thicker shells. Under the light from a 100 W halogen lamp, the transmittance through one side of each carapace shell (Fig. 1) was measured over a wavelength range of 350 nm to 850 nm at 1 nm intervals with a spectroradiometer (HSR-8100, Maki Manufacturing Co., Ltd., Hamamatsu, Japan). The transmittance spectrum was integrated to estimate the total transmittance of the carapace shell of prawn postlarvae and juveniles grown at different water pH. To determine if the total transmittance of the carapace changes with growth, the carapace of 12 postlarvae and early juveniles (13.4 - 25.0 mm TL) reared at ambient pH were scanned for transmittance spectra and the total transmittance integrated.

Data analysis

When all of the possible observations from a population falls into one of two discrete categories, such a population is called a binary population (Siegel and Castelan Jr. 1988).

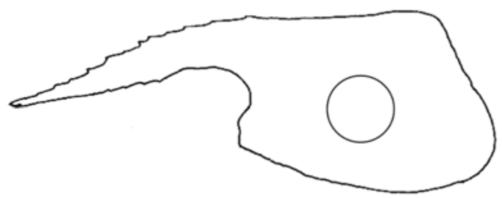

Fig. 1. Illustration of carapace showing the location (circle) for transmittance measurement by spectrophotometry

Since the survival data are from a binary population, the binomial theorem was applied to the statistical analysis of survival ratios, and 95% binomial confidence intervals of the survival ratios observed were calculated (Clopper and Pearson 1934; Soper 2014). For the statistical tests of TL, BW, and transmittance integral, the *t*-distribution based 95% confidence intervals were calculated (Snedecor and Cochran 1989). For the TL and BW frequency distributions, with different sample sizes, dispersion was evaluated by the anomaly coefficient of variance ACV, defined as the ratio of the standard error SE to the median: ACV = (SE / median) × 100.

3. Results

Survival and growth

After 27 d rearing in aquaria, survival was significantly higher (83.6%) at pH 6 than at control pH 7.5 (62.4%) and

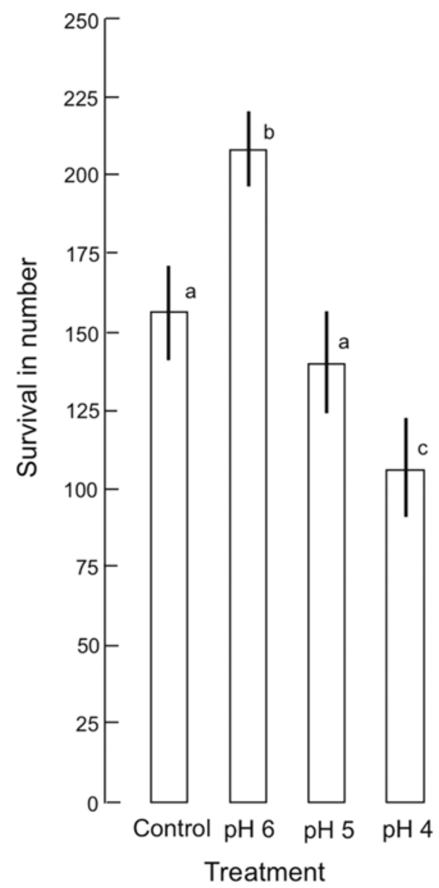

Fig. 2. Survival of *Macrobrachium rosenbergii* postlarvae and juveniles reared at pH 4 - pH 7.5 for 27 d. Aquaria were stocked with 250 postlarvae each. Vertical bars, 95% binomial confidence intervals. Different letters mark significant differences at $\alpha = 0.05$

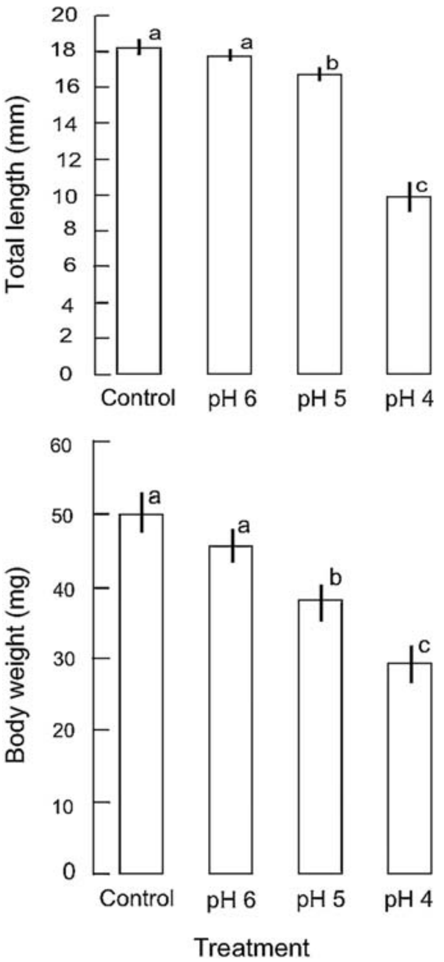

Fig. 3. Final median total length and median body weight of of *Macrobrachium rosenbergii* postlarvae and juveniles reared at pH 4 - pH 7.5. Vertical bars, 95% binomial confidence intervals. Different letters mark significant differences at $\alpha = 0.05$

lowest at pH 4 (42.4%) (Fig. 2). The 70 (66% of survival) postlarvae reared at pH 4 did not metamorphose into juveniles, but those at the higher pH did, and reached final TL and BW significantly higher at pH 7.5 and pH 6 than at pH 5 (Fig. 3). At ambient pH 7.5, *Macrobrachium rosenbergii* postlarvae metamorphose into juveniles at about 14 mm TL (Kawamura et al. submitted).

Frequency distribution of total length and body weight

The frequency distributions of the final TL and BW of *M. rosenbergii* postlarvae and juveniles reared at low pH are shown in Fig. 4 and Fig. 5. At the control pH 7.5, TL was distributed in 11 size classes and BW in 10 size classes. At pH 6, TL and BW showed typical normal distributions with 13 and 9 size classes. At pH 5, the size distributions were

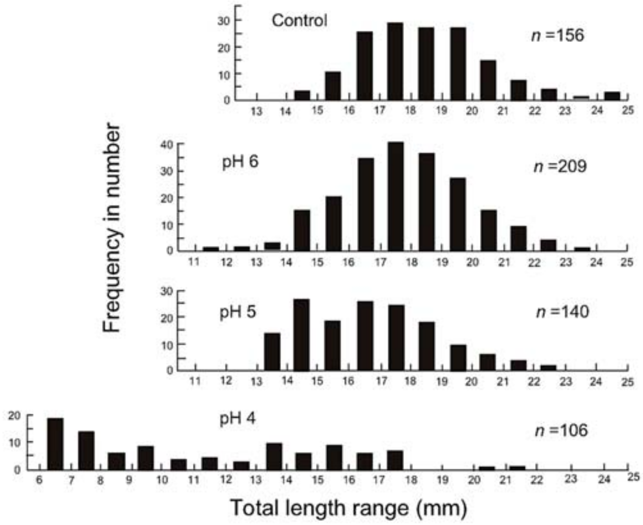

Fig. 4. Total length frequency distributions of *Macrobrachium rosenbergii* postlarvae and juveniles reared at pH 4 - pH 7.5

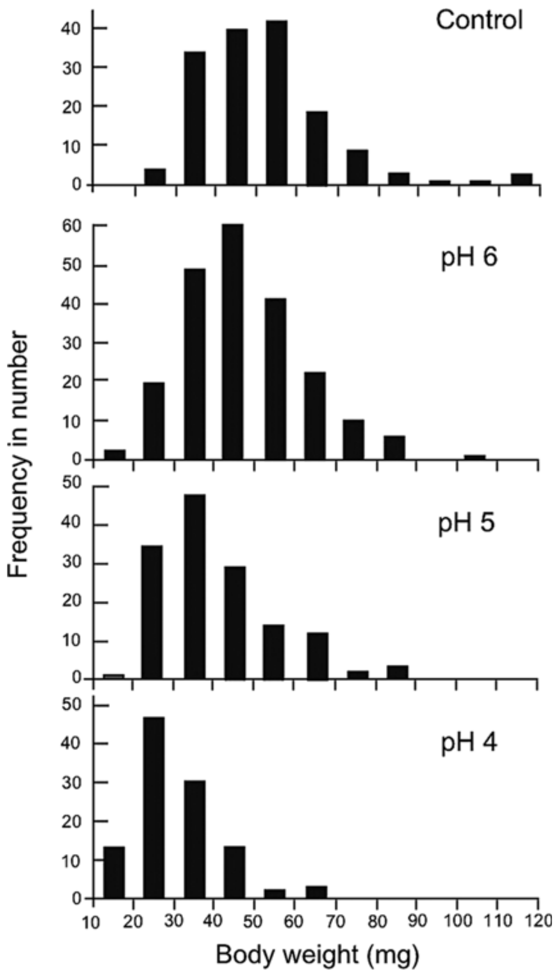

Fig. 5. Body weight frequency distributions of *Macrobrachium rosenbergii* postlarvae and juveniles reared at pH 4 - pH 7.5

Table 2. Anomaly coefficient of variance ACV and parameters for calculation

A. Total length					
pH treatment	Parameter				
	n	Median (mm)	SD (mm)	SE (mm)	ACV
Control	156	18.24	1.9946	0.1597	0.0087
pH 6	209	17.72	2.1122	0.1461	0.0082
pH 5	140	16.66	2.1566	0.1823	0.0110
pH 4	106	9.84	4.1662	0.4047	0.0412
B. Body weight					
pH treatment	Parameter				
	n	Median (mg)	SD (mg)	SE (mg)	ACV
Control	156	50.25	16.3535	1.3093	0.0261
pH 6	209	45.00	15.4126	1.0661	0.0237
pH 5	140	38.15	14.2752	1.2065	0.0316
pH 4	106	28.65	10.4844	1.0183	0.3554

n, number of specimens; SD, standard deviation; SE, standard error

shifted to the left, TL in 10 size classes and BW in only eight. At pH 4, more postlarvae were at smaller sizes, TL was widely distributed over 16 size classes, but BW in only six. The ACVs for TL and BW were smallest at pH 6 and extremely large at pH 4 (Table 2). Thus the size distributions were positively skewed at the control pH 7.5, normal at pH 6, shifted to smaller sizes at pH 5, and even smaller at pH 4.

Total length-body weight regressions

The relation between total TL and BW of *M. rosenbergii* prawn postlarvae and juveniles reared at four pH treatments is shown in Fig. 6 and represented by linear regression lines as follows:

pH 7.5 $\log BW = 2.59 \log TL - 1.57$

pH 6 $\log BW = 2.69 \log TL - 1.71$

pH 5 $\log BW = 2.49 \log TL - 1.45$

At pH 4, the relation showed a break at TL = 11.75 mm and was better represented by two linear regression lines as follows:

$\log BW = 1.13 \log TL + 0.52$ (unmetamorphosed postlarvae)

$\log BW = 2.64 \log TL - 1.66$ (juveniles)

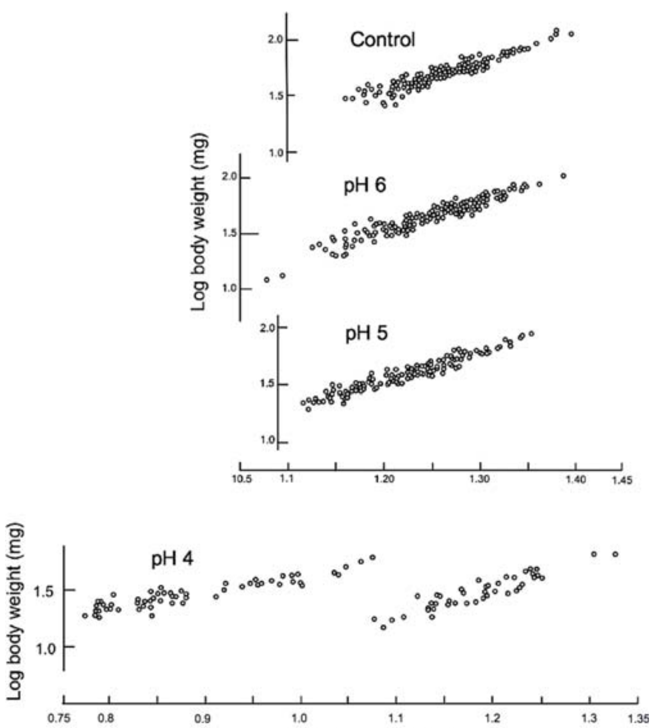

Fig. 6. Regression of log total length and log body weight of *Macrobrachium rosenbergii* postlarvae and juveniles reared at pH 4 - pH 7.5

Carapace shell transmittance spectrum and integral

Transmittance spectra of the carapace of prawn postlarvae and juveniles were of three types (Fig. 7). Spectra A and B had flat bottoms with two or three small peaks at 435 - 436 nm, 545-546 nm, and 610 - 613 nm. The carapace of juveniles that survived at pH 7.5, pH 6, and pH 5 showed spectra A and B. The carapace of the juveniles and unmetamorphosed postlarvae that survived at pH 4 showed spectrum C with increasing transmittance from 450 nm to 850 nm and no small absorption peaks (Fig. 7). Deformation of the carapace was not observed in any of the pH treatments.

The mean transmittance integrals of the carapace shells of

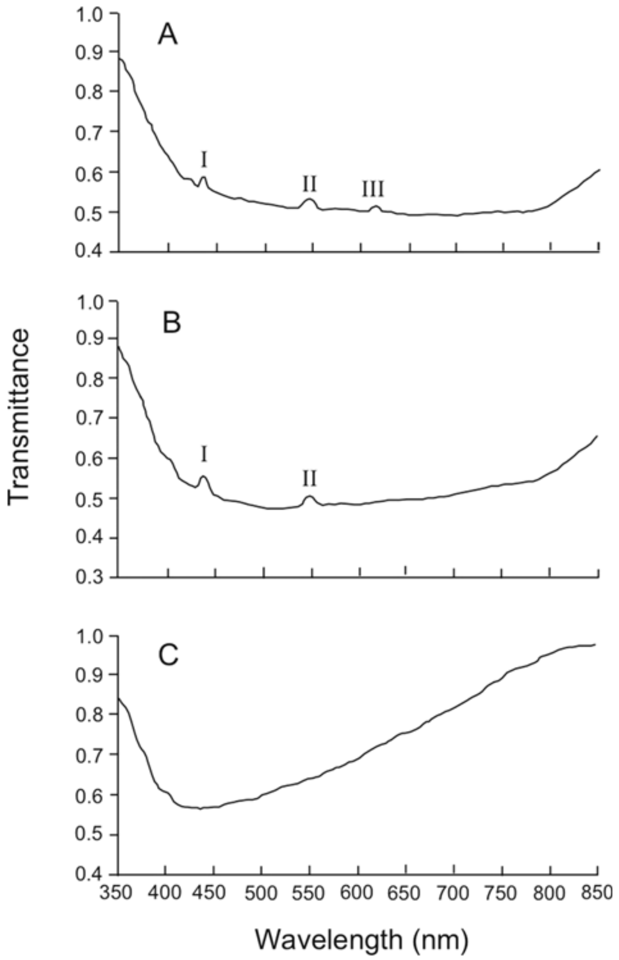

Fig. 7. Three types of transmittance spectra of the carapace of *Macrobrachium rosenbergii* reared at pH 4 - pH 7.5. Spectrum A had a flat bottom and three small absorption peaks I, II, and III. Spectrum B had a flat bottom and two small absorption peaks I, and II. Spectrum C showed increasing transmittance with wavelength and no small absorption peaks. Transmittance is given as the ratio of transmitted over the radiated light

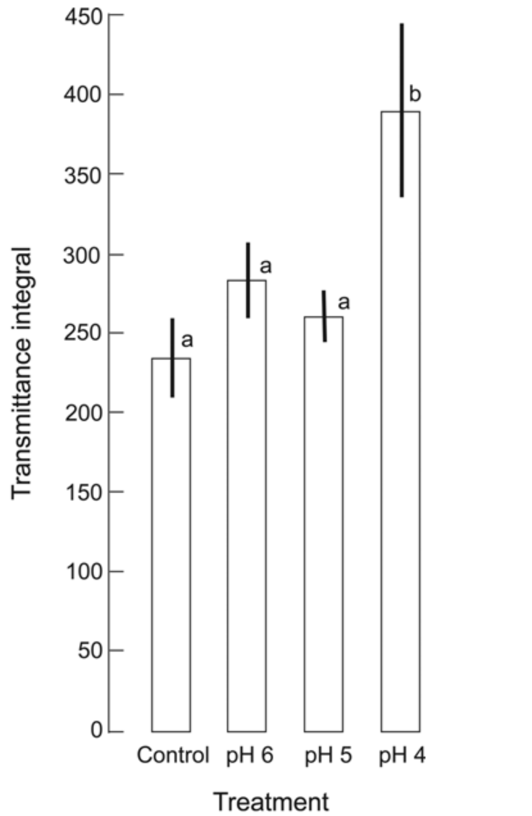

Fig. 8. Transmittance integral of the carapace of *Macrobrachium rosenbergii* reared at pH 4 - pH 7.5. Vertical bars, 95% confidence intervals. Different letters mark significant differences at $\alpha = 0.05$.

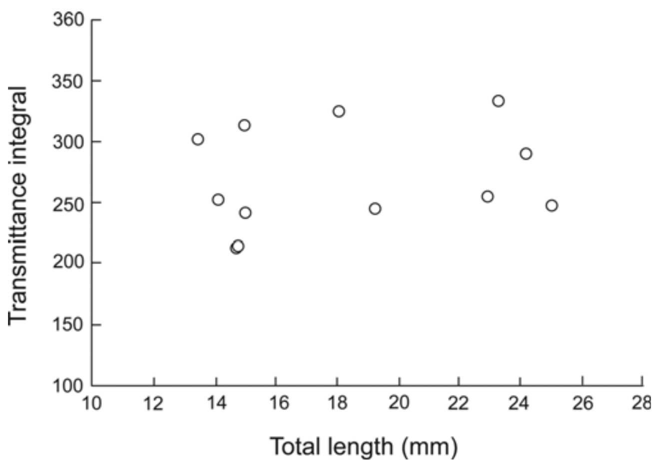

Fig. 9. Correlation between total length and carapace thickness (as transmittance integral) of *Macrobrachium rosenbergii* reared at ambient pH

specimens from the pH treatments are shown in Fig. 8. Transmittance was higher (228 - 476) through the carapace of specimens that survived at pH 4 than through the carapace of juveniles that survived at control pH 7.5, pH 6, and pH 5 (208 - 358). The mostly smaller survivors at pH 4 had thinner carapace, whereas the larger survivors at pH 5 - 7.5 had thicker carapace.

A separate batch of prawn of 13.4 - 25.0 mm TL (mostly juveniles) reared at ambient pH 7.5 showed similarly thick carapace with transmittance integral of 216 - 334, but a low correlation coefficient of $r = 0.1535$ ($t = 0.480$, $P > 0.50$; Fig. 9).

4. Discussion

Effect of low pH on survival and growth

Sudden lowering of the pH causes shock to decapod crustaceans (Almut and Bamber 2013) and may later affect survival and growth (Lemonnier et al. 2004; Tucker and D'Abramo 2008). The present experiment on *Macrobrachium rosenbergii* postlarvae determined the effect of prolonged 27 d exposure to low pH. Survival was significantly higher at pH 6 than at pH 7.5 and pH 5, and worst at pH 4. The TL, BW, size frequency distributions and TL-BW regressions of the survivors indicate better growth at pH 7.5 and pH 6. Chen and Chen (2003) reared *M. rosenbergii* juveniles for 56 d at pH 8.2, 7.4, 6.8, 6.2, and 5.6 in the laboratory and observed respective survival ratios of 100%, 88.9%, 94.4%, 94.4%, and 94.4%, and significantly lower BW and TL at pH 5.6; they concluded that the minimum acceptable pH levels were 6.2 and 7.4 based on growth and feeding. Our results are consistent with those of Chen and Chen (2003). Our experiment did not cover pH > 7.5 because we focused on the effects of low pH, below the environmental pH range 6.0 - 8.5 of natural waters (Tucker and D'Abramo 2008).

The pH range 7.0 - 8.5 is known to be optimum for *M. rosenbergii* growth (New 1995) and the haematolymph of postlarvae is at pH 7.60 (Yeh et al. 2006). The pH of the external medium affects the osmolality of *M. rosenbergii* (Chen and Kou 1996) and osmoregulatory cost is minimized in an optimum isosmotic medium such that energy savings can be diverted to fish growth (Boeuf and Payan 2001). The experimentally determined best pH in this study, pH 6, is outside the known optimum range but within the environmental pH range 6.0 - 8.5 of natural waters (Tucker and D'Abramo 2008). Since rearing water pH 6 is quite different from haematolymph pH 7.6, some other non-osmoregulatory mechanism must be responsible for higher growth and survival at pH 6 than at ambient pH 7.5. Cheng and Chen (1998) reported that *Enterococcus*-like infection in *M. rosenbergii* was exacerbated by high pH (pH 8.8 - 9.5) but reduced by low salinity and lower pH (pH 7.5 - 7.7 and pH 4.6 - 5.2). These data are consistent with our better results at pH 6.

Growth heterogeneity

Populations of *M. rosenbergii* postlarvae have a relatively homogenous size distribution and rapidly increase in size variation after stocking in ponds (Karplus et al. 2000). The wide disparity in size among a cohort of *M. rosenbergii* is a major bottleneck in its successful aquaculture (Ranjeet and Kurup 2002). *M. rosenbergii* is aggressive and cannibalistic (Segal 1975; Nair et al. 1999) and postlarvae and juveniles are susceptible to intraspecific attacks during moult and postmoult stages when stocked at high density (Alston and Sampaio 2000). Size variation is thought to be the major cause of cannibalism (Hecht and Pienarr 1993). It is common practice to do size grading of *M. rosenbergii* juveniles before stocking them in grow-out ponds (New 2002), but only a slight effect of size grading was seen among *M. amazonicum* juveniles in ponds (Preto et al. 2010). In the present study, TL and BW frequency showed normal distribution at pH 6, positive skew at pH 7.5 and pH 5, and extremely wide scatter at pH 4. Given that ACV was lowest at pH 6, a wide scatter in sizes seemed to be minimized by rearing *M. rosenbergii* postlarvae at pH 6.

Carapace shell quality

The effect of low pH on the shell quality of *M. rosenbergii* postlarvae and juveniles was assessed by spectrophotometry for the first time in this study. Changes in the shell quality may be secondary to the adverse effect of pH 4 on growth and metamorphosis. The higher transmittance (thus, decreased thickness) and the absence of the small peaks at 436 nm, 545 nm, and 612 nm in the spectra of the carapace indicate a different shell quality at pH 4. The crustacean exoskeleton is a sophisticated structure of chitin and calcium carbonate (Boβelmann et al. 2007). Under very low pH, the shells of clams, oysters, and some snails and urchins partially dissolve (Madin 2010) but crustaceans do not seem to be harmed and even appear to increase their exoskeleton-building (Madin 2010). The gill of *Metapenaeus* shrimp functions as a simple membrane and absorbs 90% of total Ca uptake (Dall 1965). In the velvet swimming crab *Necora puber*, any extracellular acidosis was completely compensated for by an increase in bicarbonate supplied by dissolution of the exoskeleton in order to maintain acid balance in the body fluid (Spicer et al. 2007). If the dissolution changes the chitin composition in the shell, it has an immunological effect on the animal. The ability of chitin to protect shrimps against pathogenic infection is well known (Song and Huang 1999; Wang and Chen 2005). Since the extent of tolerance to low pH in crustaceans is species specific (Kurihara and Ishibashi 2008), the results of the present study should not be extrapolated to other crustacean species.

5. Conclusions

Survival, growth, size distribution, and carapace quality of *M. rosenbergii* postlarvae and early juveniles were negatively affected by pH 5 and especially pH 4. Natural waters affected by acid rain could decimate *M. rosenbergii* populations in the wild. Hatchery rearing, nursery, and growout of *M. rosenbergii* are best done at pH 6 - 7.5.

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