Effects of salinity and alkalinity on growth and survival of all-male giant freshwater prawn (*Macrobrachium rosenbergii* De Man, 1879) juveniles

Pham Van Day[1], Huynh Kim Huong[1], Phan Thi Thanh Truc[1], Nguyen Thi Hong Nhi[1], Diep Thanh Toan[1], Lai Phuoc Son[1], Ho Khanh Nam[1], Pham Thi Binh Nguyen[1], Phan Chi Hieu[1], Doan Xuan Diep[2,*] and Le Thi Phuong Mai[3]

[1] *School of Agriculture and Aquaculture, Tra Vinh University, Tra Vinh Province, Vietnam*
[2] *Medicinal Chemistry, Hi-tech Agriculture & Bioactive Compounds Research Group, School of Engineering and Technology, Van Lang University, Ho Chi Minh City, Vietnam*
[3] *College of Rural Development, Can Tho University, Can Tho City, Vietnam*

Abstract

All-male giant freshwater prawns (AMGFPs) have been a popular crop cultivated in the Mekong Delta, Vietnam, due to their proven production efficiency compared to all-female or mixed-sex prawn cultures. However, the crucial water quality factors impacting AMGFP aquaculture efficiency have yet to be elaborately investigated. Two separate experiments were randomly arranged with three replicates to evaluate the effects of salinity or alkalinity on the growth and survival of AMGFP juveniles during the grow-out period. The results show that the prawn survival rate in the salinity range of 0–15‰ varied from 66.1 to 74.8% and in a salinity range of 0–5‰ was relatively low compared to the range of 10-15‰; however, the difference was not significant among salinities after 90 days of culture ($p > 0.05$). All the prawn growth performance parameters significantly decreased with increasing salinities of 0, 5, 10, and 15‰ after 30, 60, and 90 days of culture ($p < 0.05$). Notably, the prawn yield did not significantly differ between salinities of 0 and 5‰ ($p > 0.05$), and both were significantly higher than those at salinities of 10 and 15‰ ($p < 0.05$) after 90 days of culture. In addition, the survival rate reached 82.5–84.4% and did not significantly differ among alkalinities of 80, 100, 120, 140, and 160 $mgCaCO_3$ L^{-1}. However, the growth performance parameters and yield of AMGFPs at an alkalinity of 160 mg L^{-1} were significantly higher than those at lower alkalinities (80, 100, 120, and 140 $mgCaCO_3$ L^{-1}) after 90 days of culture. Therefore, it is recommended that a salinity range of 0–5‰ and alkalinity of 160 $mgCaCO_3$ L^{-1} is optimal for the growth-out culture of AMGFP juveniles.

* Corresponding author. Doan Xuan Diep, diep.dx@vlu.edu.vn

Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii* De Man 1879) is an economically important aquaculture species in many countries around the world (FAO, 2022; New, 1995; New & Nair, 2012), particularly in South and Southeast Asia (New & Valenti, 2008), due to culturing advantages such as large body size, fast growth, short culture period, and good disease resistance. Additionally, they are suitable for tropical climates and have widely available global markets (FAO, 2019). In 2019, *M. rosenbergii* accounted for 45% of global cultured freshwater prawn output, totaling 0.26 million tons worth $2.01 billion, with Vietnam accounting for 20,129 tons, ranking fourth behind China (139,609 tons), Bangladesh (52,197 tons), and Thailand (31,345 tons) (FAO, 2022)

Previous studies confirmed that the efficiency of AMGFP culture was higher than that of all-female or mixed-sex cultures (Brody et al., 1980; Cohen et al., 1988; Levy et al., 2017; Nair et al., 2006; Siddiqui et al., 1997; Tidwell et al., 2015) because males have a faster growth rate and a larger maximum body size than females. Moreover, after maturation, females nearly stop growing (Ra'Anan et al., 1991) as a large portion of the energy is allocated to the development of the reproductive system and egg production (Siddiqui et al., 1997; Tidwell et al., 2015). Therefore, many studies focused on giant freshwater prawn transgender issues in the direction of all-male prawn production to improve the productivity of farmed prawns (Malecha et al., 1992; Ohs et al., 2006; Sagi et al., 1986; Siddiqui et al., 1997; Ventura et al., 2009; Ventura et al., 2012; Lezer et al., 2015; Ha, 2014).

Furthermore, *M. rosenbergii* naturally inhabits most inland freshwater areas such as rivers, lakes, swamps, canals, ponds, and estuarine regions (Davassi, 2011). The reproductive females migrate to estuaries for spawning, where the larvae grow in brackish water. After metamorphosis into postlarvae, they migrate upstream and grow to reach the maturation stage in freshwater habitats. By mating season, *M. rosenbergii* will return to estuaries to spawn in brackish water (New, 2002). With this lifestyle, salinity significantly impacts the stages of their lives. Additionally, alkalinity is a vital water variable for the culturing system of *M. rosenbergii* (Biesterfeld et al., 2003; Chen et al., 2006; Colt, 2006; Paz, 1984). In aquatic environments, alkalinity and pH in water chemistry are closely related. Drastic changes in pH could disrupt physiological functions and interfere with the growth of aquatic organisms. Alkalinity is a buffer to stabilize the water's pH (Boyd, 2016; Boyd et al., 2016). Furthermore, the total hardness and alkalinity of the aquatic medium are crucial considerations in *M. rosenbergii* culture because they represent the concentrations of cations (calcium-magnesium) and anions (carbonates) required for crustacean exoskeleton mineralization (Boyd, 1979; Boyd & Tucker, 1992).

For more evidence regarding the effect of salinity and alkalinity on the development of AMGFPs to support this prawn farming development in a context where the intrusion of salt into inland areas is increasing widely due to the impact of climate change worldwide (Nielsen & Brock, 2009; Williams, 2001), particularly in Vietnam's Mekong Delta, where climate change can increase salinity on average by 2–2.5‰ and add roughly 6% additional salinity-affected areas by 2040 in all coastal provinces by 2040, and increase the total affected area by 10–25% by 2050 (Eslami et al., 2021). Our study aims to investigate the production efficiency of AMGFP juveniles in terms of growth performance, survival rate, and yield at various salinities and alkalinities during the grow-out period. The findings are the basis for expanding AMGFP farming in the Mekong Delta in Vietnam and other countries where the AMGFP farming industry is developing.

Materials and Methods

Experimental materials

The postlarvae of AMGFPs (PLs) were sourced from artificial reproduction by pseudo-female broodstock of *M. rosenbergii* at the Aquaculture Breeding Center of Tra Vinh Province in Southern Vietnam using RNAi technology developed by Ventura et al. (2009), Ventura et al. (2012), and Ha (2014). The PLs were then moved to the Experimental Aquaculture Hatchery of Tra Vinh University. Here the prawns were nursed in composite

tanks (volume of 4 m^3 with 3.5 m^3 of water per tank) with a biofloc system by Avnimelech (2012) for two months at a density of 500 ind·m^{-3} with a pellet feed (40% of crude protein). The salinity levels were 0, 5, 10, and 15‰ (for experiment 1), and alkalinity levels were 80, 100, 120, 140, and 160 $mgCaCO_3$ L^{-1} (for experiment 2) in the tanks where the PLs were kept until reaching the juvenile stage. At the end of the nursing periods, the AMGFP juveniles of each salinity or alkalinity were collected and arranged in the respective experimental units.

Freshwater and saltwater were the two types of water used in the experiments. The freshwater was taken from the river, and the sediment was removed. The water was then disinfected with potassium permanganate ($KMnO_4$) at a dosage of 5 mg L^{-1} and continuously aerated for 1 to 3 days. The saltwater with a salinity of 120‰ was treated with 30 mg L^{-1} chlorine and continuously aerated for 3 to 4 days. The two sources of water were then mixed to achieve the investigated salinities. The water was pumped into the experimental tanks through a filter bag with a mesh size of 5 μm. Grobest pellet feed (made in Vietnam) contains 35–40% protein with a particle size of 0.1–1.2 mm, which was used in two experiments, depending on the development stage of the prawns. The composite tanks were used in this study. The 4 m^3 tanks were used for the nursing period, and the 6 m^3 tanks were used for the grow-out culture period.

Experimental design

This study was conducted at the Aquaculture Experimental Hatchery of Tra Vinh University, Vietnam, from May to July 2021. Two separate completely randomized experiments, with three replicates, aimed to look into the effects of salinity or alkalinity on the growth and survival rates of AMGFP juveniles during their growing out, including:

The first experiment investigated four salinity levels of 0, 5, 10, and 15‰. The AMGFP juveniles after two months of nursing (mean weight of 1.23 g and mean length of 5.12 cm) were arranged randomly in twelve 6m^3 composite tanks containing 5.5 m^3 of water with corresponding salinities (three tanks for a salinity) at a density of 15 ind m^{-3}. The prawns were fed 4–7% of their body weight, depending on the development stage of the prawns, three times daily at 6:00, 11:00, and 16:00. The water in the tanks was constantly aerated to ensure dissolved oxygen demand for the prawns during the culture duration. Fecal matter and uneaten feed in tanks were siphoned every day at 21:00. Before siphoning, all the aeration faucets of the tanks were turned off so that waste would settle at the bottom of the tanks. The water in culturing tanks was changed by 20–30% every ten days. The experimental time lasted 90 days.

In the second experiment, five alkalinity levels of 80, 100, 120, 140, and 160 $mgCaCO_3$ L^{-1} were investigated at a salinity of 0‰ during the AMGFP grow-out culture for 90 days. Before stocking the prawns, the water alkalinity in each experimental unit was adjusted according to the method of Furtado et al. (2015) and measured by the standard method of APHA (1989). The initial water source had an alkalinity level of 128 $mgCaCO_3$ L^{-1}, and 1 M hydrochloric acids (HCl) (Synth®) was added to reach the desired alkalinity levels of 80, 100, and 120 $mgCaCO_3$ L^{-1}. Sodium bicarbonate ($NaHCO_3$) with 99% purity (Carbonor®) was added for alkalinity levels of 140 and 160 $mgCaCO_3$ L^{-1}. Throughout the experiment, various dosages of sodium bicarbonate were added to maintain the trial alkalinity levels (<0.15 g/L per application). The pH values in treatment aqueous solutions were checked and ranged from 8.19 to 8.38, increasing gradually as alkalinity increased. The AMGFP juveniles (mean weight of 1.51 g and mean length of 5.61 cm) were stocked in 15 composite tanks of 6 m^3 with 5.5 m^3 of water per tank (three tanks for each alkalinity) at a density of 15 ind m^{-3}. The prawn care and experimental management processes in this experiment were the same as in the first experiment.

Data collection and calculation

In both experiments, fifteen individual prawns were randomly collected to determine growth performance parameters immediately before being arranged in the tanks and every 30 days during the experimental period. At each sampling, each organism was gently

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blotted dry and weighed individually to the nearest 0.001 g using a digital balance (Mettler AJ100). Simultaneously, the length was measured to the nearest millimeter from the rostrum tip to the telson end using a divided ruler. The prawn survival rate and yield were recorded and calculated at the end of the experiments.

Water quality was controlled right before and during the cultivated period, including temperature and pH, which were measured twice a day by a thermometer and pH meter, at 7:00 and 14:00; nitrite, alkalinity in experiment 1, and TAN were checked every three days at 14:00 using the Sera GH test kits (Germany).

The growth performance parameters, survival rate, and yield were calculated using the following equations:

Daily weight gain/DWG (g day^{-1}) = final weight − initial weight/culturing days
Daily length gain/DLG (cm day^{-1}) = final length − initial length/culturing days
Specific growth rate in weight/SGR$_W$ (% day^{-1}) = [(Ln final weight) − (Ln initial weight)]/culturing days × 100
Specific growth rate in length/SGR$_L$ (% day^{-1}) = [(Ln final length) − (Ln initial length)]/culturing days × 100
Survival rate (%) = (final prawn number/initial prawn number) × 100
Yield (g m^{-3}) = prawn biomass/culture volume

Statistical analysis
Levene's test assessed the variance homogeneity, and the percentage data were transformed to arcsine before conducting statistics. One-way analysis of variance (ANOVA) was conducted on all data, followed by the Duncan test to identify significant differences between the mean values (significance level of $p < 0.05$) using the Statistical Package for the Social Sciences (SPSS) software for Windows version 20.0.

Results

Water quality parameters
The water quality parameters monitored in both experiments fluctuated within the suitable range for *M. rosenbergii* and were not significantly different between the culturing tanks during periods of culture ($p > 0.05$). In the first experiment, the following ranges were observed: temperature of 26.83–27.83°C, pH of 8.50–8.54, TAN of 0.19–0.22 mg L^{-1}, nitrite of 1.15–2.36 mg L^{-1}, and alkalinity of 128.1–139.3 mg L^{-1}. In the second experiment, the following ranges were observed: temperature of 27.3–28.0°C, pH of 8.19–8.39, TAN of 0.19–0.56 mg L^{-1}, and nitrite of 0.08–0.18 mg L^{-1}.

Growth and survival of AMGFP juveniles at various salinities

The growth performance
Data in **Figures 1-2** show that the initial mean size (weight of 1.23–1.49 g and length of 5.12–5.47 cm) of the AMGFPs was not significantly different in the investigated salinity range of 0–15‰ ($p > 0.05$). However, after 30, 60, and 90 days of culture, the mean weight and length decreased with increased salinity; there was always a significant difference between salinities of 0, 5, 10, and 15‰ at the sample times ($p < 0.05$). The highest mean weight and length obtained at a salinity of 0‰ were 5.34, 12.51, and 17.23 g; and 8.24, 10.51, and 11.72 cm; while the lowest mean weights and lengths obtained at a salinity of 15‰ were 2.14, 1.49, and 7.33 g; and 6.27, 7.78, and 8.77 cm at the sampling times on the 30, 60, and 90th culturing days, respectively. Remarkably, at the end of the time of culture, the mean weight of the prawns significantly decreased at salinities of 10 and 15‰, which were calculated to be only equivalent to 59.8 and 42.5% compared to that at the salinity of 0‰, respectively.

In a similar variation pattern in the mean size, the parameters of daily weight gain (DWG) and specific growth rate in weight (SGR$_W$), as well as daily length gain (DLG) and

specific growth rate in length (SGR$_L$) of AMGFPs decreased as salinity increased. There was always a significant difference between the salinities of 0, 5, 10, and 15‰ after 90 days of culture ($p < 0.05$). The highest values at a salinity of 0‰ were 0.18 g day^{-1}, 2.87% day^{-1}, 0.07 cm day^{-1}, and 0.92% day^{-1}; and the lowest values at a salinity of 15‰ were 0.06 g day^{-1}, 1.65% day^{-1}, 0.04 cm day^{-1}, and 0.51% day^{-1}, respectively at the end of the experiment (**Table 1**).

Survival rate and yield

Results in **Table 2** showed that the survival rate of AMGFPs after 90 days of culture in a salinity range of 0–5‰ was relatively low compared to the range of 10-15‰. The highest survival rate (73.3%) was obtained at a salinity of 10‰ and the lowest (66.1%) at a salinity of 0‰, although differences were not significant in the investigated salinity range ($p > 0.05$).

The yield of AMGFPs after 90 days of culture was presented in **Table 2**, which showed that this parameter was not significantly different between salinities of 0 and 5‰ ($p > 0.05$). Both were significantly higher than those at 10 and 15‰ ($p < 0.05$), with descending values of 174.9, 155.5, 112.1, and 80.2 g m^{-3}, respectively, to salinities of 0, 5, 10, and 15‰.
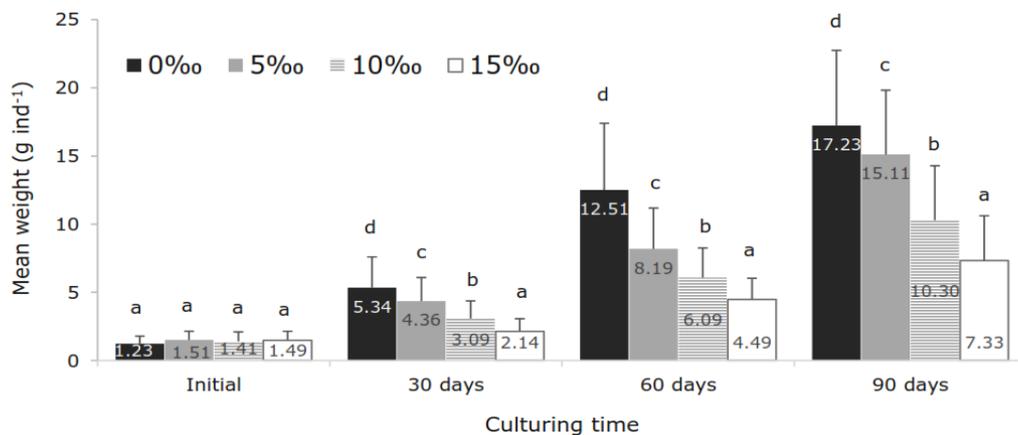
Figure 1 Mean weight of AMGFPs during 90 days of culture at various salinities
[Values are presented as mean±SD. The different bars with different letters (a, b, c, d) at the same sampling time in Figure 1 show a significant difference ($p < 0.05$)]
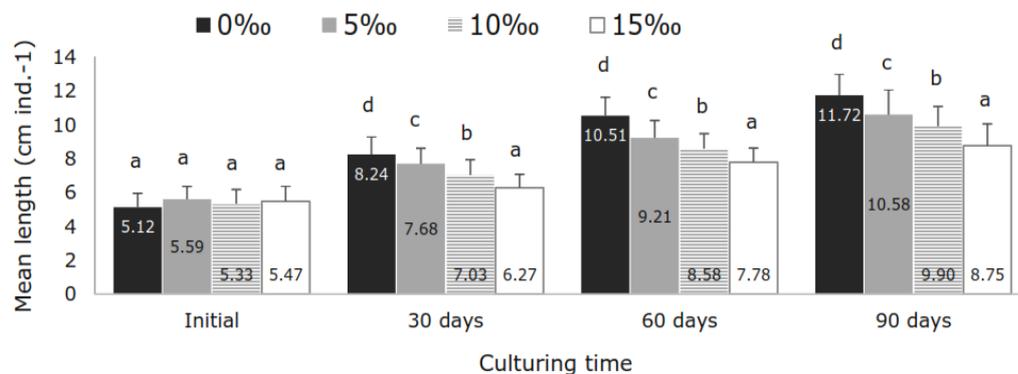
Figure 2 Mean length of AMGFPs during 90 days of culture at various salinities
[Values are presented as mean±SD. The different bars with different letters (a, b, c, d) at the same sampling time in Figure 2 show a significant difference ($p < 0.05$)]

Table 1 Daily weight gain (DWG), specific growth rate in weight (SGR$_W$), daily length gain (DLG), and specific growth rate in length (SGR$_L$) of AMGFPs after 90 days of culture at various salinities

Salinities (‰)	DWG (g day^{-1})	SGR$_W$ (% day^{-1})	DLG (cm day^{-1})	SGR$_L$ (% day^{-1})
0	0.18±0.06[d]	2.87±0.39[d]	0.07±0.01[d]	0.92±0.12[d]
5	0.15±0.05[c]	2.52±0.34[c]	0.06±0.02[c]	0.7±0.16[bc]
10	0.10±0.04[b]	2.12±0.46[b]	0.05±0.01[b]	0.68±0.14[b]
15	0.06±0.04[a]	1.65±0.54[a]	0.04±0.01[a]	0.51±0.17[a]

Values are presented as mean±SD. Values with different letters (a, b, c, d) in the same column show a significant difference (p < 0.05).

Table 2 Survival rate and yield of AMGFPs after 90 days of culture at various salinities

Salinities (‰)	0	5	10	15
Survival rate (%)	66.1±6.3[a]	69.4±5.1[a]	73.3±4.4[a]	72.8±3.5[a]
Yield (g m^{-3})	174.9±17.[c]	155.5±21.9[c]	112±9.1[b]	80±9.7[a]

Values are presented as mean±SD. Values with different letters (a, b, c) in the same row show a significant difference (p < 0.05).

Growth and survival rates of AMGFP juveniles at various alkalinities

The growth performance

Results presented in **Tables 3-4** indicate that the AMGFPs had a mean weight ranging from 4.14 to 5.03 g and a mean length ranging from 9.15 to 11.08 g and were not significantly different in investigated alkalinity range of 80–160 mgCaCO$_3$ L^{-1} (p > 0.05) at the commencement of the experiment. In the first 60 days, the effect of various alkalinities was not apparent on the mean weight and length of the prawns in the 80–160 mgCaCO$_3$ L^{-1} solutions. The lack of significant difference in these parameters at alkalinities of 80, 100, 120, 140, and 160 mgCaCO$_3$ L^{-1} (p > 0.05). However, after 90 days of culture, both MW and ML, as well as DWG, SGRw, DLG, and SGR$_L$ of the prawns, were the highest at an alkalinity of 160 mgCaCO$_3$ L^{-1}, with values of 17.18 g, 11.07 cm, 0.17 g day^{-1}, 2.60 ‰ day^{-1}, 0.06 cm day^{-1}, and 0.73‰ day^{-1}, respectively (p < 0.05), which is significantly different from those at lower alkalinities. However, these parameters in the alkalinity range of 80–140 mgCaCO$_3$ L^{-1} varied from 11.35 to 11.45 g ind^{-1}, 10.04 to 10.34 cm ind^{-1}, 0.11 g day^{-1}, 2.12 to 2.28‰ day^{-1}, 0.05 cm day^{-1} and 0.64 to 0.68‰ day^{-1}, respectively and there was not a significant difference in this alkalinity range.

Survival rate and yield

The prawn survival and yield were documented and calculated after 90 days of culture. **Figures 3-4** show that the survival rates did not vary significantly in the alkalinity range of 80 to 160 mgCaCO$_3$ L^{-1}, ranging from 82 to 84‰. The highest prawn yield (172.4 g m^{-3}) was obtained at an alkalinity of 160 mgCaCO$_3$ L^{-1}, which differed significantly from yields obtained at lower alkalinity levels. The prawn yield at the alkalinity range of 80–140 mgCaCO$_3$ L^{-1} varied from 131.1 to 141.1 g m^{-3}; there were no significant differences in this alkalinity range.

Table 3 Initial mean weight (IMW), mean weight (MW), daily weight gain (DWG), and absolute growth rate in weight (SGRw) of AMGFPs during 90 days of culture at various alkalinities

Parameters	Alkalinities (mgCaCO$_3$ L^{-1})				
	80	100	120	140	160
IMW (g ind^{-1})	1.51±0.57[a]	1.62±0.10[a]	1.41± 0.36[a]	1.42±0.22[a]	1.62±0.20[a]
MW30 (g ind^{-1})	4.70±1.95[a]	4.21±2.13[a]	4.27±1.89[a]	4.14±1.0[a]	5.03±1.78[a]
MW60 (g ind^{-1})	10.52±4.93[a]	9.15±3.63[a]	9.47±4.67[a]	9.27±4.64[a]	11.08±4.36[a]
MW90 (g ind^{-1})	11.35±3.51[a]	11.23±2.63[a]	11.45±3.18[a]	11.40±3.29[a]	17.18±3.65[b]
DWG (g day^{-1})	0.11±0.04[a]	0.11±0.03[a]	0.11±0.04[a]	0.11±0.04[a]	0.17±0.04[b]
SGRw (‰ day^{-1})	2.19±0.36[ab]	2.12±0.28[a]	2.28±0.31[b]	2.27±0.31[b]	2.60±0.25[c]

Values are presented as mean±SD. Values with different letters (a, b, c) in the same row show a significant difference (p < 0.05).

Table 4 Initial mean length (MIL), mean length (ML), daily length gain (DLG), and absolute growth rate in length (SGR$_L$) of AMGFPs during 90 days of culture at various alkalinities

Parameters	Alkalinities (mgCaCO$_3$ L^{-1})				
	80	100	120	140	160
IML (cm ind^{-1})	5.61±0.61[a]	5.67±0.72[a]	5.56±0.42[a]	5.57±0.46[a]	5.69±0.45[a]
ML30 (cm ind^{-1})	7.88±1.04[ab]	7.68±1.06[a]	7.79±1.05[ab]	7.57±1.13[a]	8.19±0.96[b]
ML60 (cm ind^{-1})	9.95±1.81[a]	9.59±1.05[a]	9.61±1.27[a]	9.63±1.36[a]	10.07±0.99[a]
ML90 (cm ind^{-1})	10.04±1.00[a]	10.13±0.80[b]	10.34±1.11[b]	10.20±0.98[ab]	11.07±1.00[c]
DLG (cm day^{-1})	0.05±0.01[a]	0.05±0.01[a]	0.05±0.01[a]	0.05±0.01[a]	0.06±0.01[b]
SGR$_L$ (% day^{-1})	0.64±0.11[a]	0.64±0.09[a]	0.68±0.11[a]	0.64±0.11[a]	0.73±0.10[b]

Values are presented as mean±SD. Values with different letters (a, b, c) in the same row show a significant difference (p < 0.05).
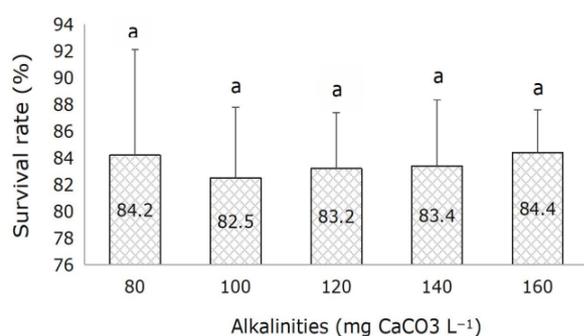
Figures 3 Survival rate of AMGFPs after 90 days of culture at various alkalinities
[Values are presented as mean±SD. The bars with different letters (a, b) in Figure 3 show a significant difference (p < 0.05)]
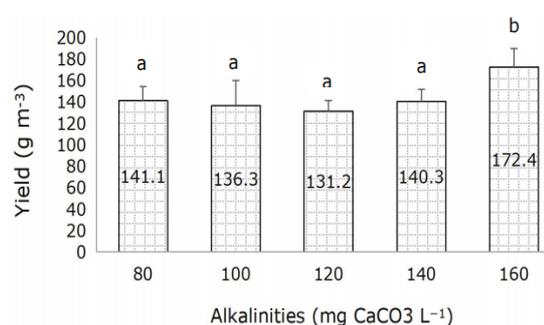
Figure 4 Yield of AMGFPs after 90 days of culture at various alkalinities
[Values are presented as mean±SD. The bars with different letters (a, b) in Figure 4 show a significant difference (p < 0.05)]

Discussion

Water quality parameters

Generally, monitored water quality factors, including temperature, pH, nitrites, TAN, and alkalinity in both experiments, were within the optimal range for prawn culture (Boyd & Zimmermann, 2000; Cheng et al., 2003; Habashy & Hassan, 2011; Huong et al., 2010; Huong et al., 2015; Mallasen et al., 2003; New, 2002; Straus et al., 1991; Wickins & Daniel, 2002). Therefore, they did not significantly affect the main research results.

Growth and survival rates of AMGFP at various salinities

In the present study, the survival rate of AMGFPs, with an initial weight of 1.23–1.51 g and a final weight of 7.33–19.08 g after 90 days of culture, showed a lower salinity range (0–5‰) was relatively low compared to the higher range (10–15‰), despite the lack of significant difference between them (p>0.05) (**Table 2**). A previous study by Huong (2015) also found similar results on a culture of the mixed-sex *M. rosenbergii* equivalent in size to the prawns in our study. They have an initial weight of 0.42–0.47 g and a final weight of 7.29–11.4 g in the salinity range of 0–15‰; the lowest prawn survival rate was at a salinity of 0‰. However, a study by Huong et al. (2010) in the subadult to adult stage (initial weight of 11.2–0.3 g and final weight of 25.6–30 g) of mixed-sex stock *M. rosenbergii* showed that both the growth and the survival of the prawns were similar in the range of 0–15‰. This difference may be due to the size of the prawns in the study by Huong et al. (2010) being larger than that in current study or Huong (2015).

The lower AMGFP survival rates at the lower salinities of the present study seemed to be associated with energy expenditure for osmotic regulation and slow shell hardness of AMGFPs in these salinities. Edwards (1982) emphasized that, under salinity changes in habitats, many aquatic animal species have the regulative ability to alter the ion

concentration and osmotic pressure of body fluids to suit those in the external environment. These regulatory mechanisms must expend energy when salinity changes are beyond the regulative ability of an animal, which could lead to its death. Previous studies indicated that the iso-osmotic salinity of *M. rosenbergii* is 14–15‰ and they are osmoregulators in freshwater up to salinities at the iso-osmotic point, whereas they are osmoconformers at higher salinities (15–28‰) (Cheng et al., 2003; Funge-Smith et al., 1995; Stern et al., 1987). Lockwood (1962) suggested that when crustaceans live in an isotonic environment, the process of water absorption and dehydration of their bodies is always in a balanced ratio, so they do not need to expend energy to push water or salt out of their bodies. Woo & Kelly (1995) found that, in freshwater conditions, aquatic species spend a certain amount of energy to compensate for the salt lost through passive diffusion. It has been argued that osmoregulation costs are the lowest when animals are iso-osmotic with their surroundings (Spaargaren, 1975). Subadult *M. rosenbergii* in the grow-out phase (19.5–19.8 g) shows a slightly reduced standard metabolic rate in 15‰ salinity compared with fresh water (Ern Andersen, 2009).

In addition to the physiological stress, the survival rate of *M. rosenbergii* can be affected in lower salinities due to increased energy expenditure, protein sparing, and depletion of lipid reserves, which in turn affect the survival rate when compared to those reared in higher salinities. It may be proven that osmoregulation in crustaceans requires energy in the form of protein (Rosas et al., 1999; Setiarto et al., 2004; Silvia et al., 2004) and lipids (Lemos et al., 2001; Sang & Fotedar, 2004). Furthermore, water with low salinity, especially groundwater, will lack the content to balance the ratio of minerals necessary for the building of their bodies (Na^+, K^+, Mg^{2+}, Ca^{2+}, and Cl^-) (Libes, 1992). Therefore, the prawns are easily trapped while molting or attacked by predators and are more susceptible to fluctuations in environmental factors and pathogens because they are slow to harden post-molt. Perhaps these were also the reasons for this study's low survival rate of AMGFPs at low salinities. AMGFPs are known to inherently have large exoskeletons, especially their claws, which require a high mineral content for shell hardening during molting. Diep (2012) also discovered the pattern of a decrease in survival with salinity decrease on the fingerling of black tiger shrimp with an isotonic salinity of 26‰, indicating that the highest survival rate was at the salinity range of 15–25‰, which was near the isotonic salinity. The lowest survival rate was at a salinity of 3‰. Diep (2012) reported that the black tiger shrimp cultured at low salinity (3‰) often in the presence of molt-trapped or slow shell hardened after molting, resulting in a low survival rate.

Contrary to the observation results of survival rate in the current study, the growth performance parameters of the AMGFPs varied with a tendency to decrease as salinity increased in the salinity range of 0–15‰. Additionally, they always significantly differed between salinities of 0, 5, 10, and 15‰ at the sampling times ($p < 0.05$) (**Figures 1-2** and **Table 1**). These results are consistent with the growth performance of the mixed-sex cultured giant freshwater prawn in previous studies (Nair & Salin, 2012; Singh, 1980; Yen & Bart, 2008). Nair & Salin (2012) reported better production, individual size, and survival of the stock of *M. rosenbergii* observed at a salinity of 5‰. Singh (1980) demonstrated that freshwater prawns could grow in salinities up to 17‰, with the highest growth achieved at salinities between 0 and 2‰. Yen & Bart (2008) cultured *M. rosenbergii* with a high stocking density; they reported that maximum growth of the prawn was at 0‰ and decreased as salinity increased, and halted growth was documented at 18‰. (Malecha, 1983) also found that although freshwater prawns tolerate higher salinities than their iso-osmotic point (18‰), optimum growth conditions are in fresh or slightly brackish water (0–4‰). New (2002) found that *M. rosenbergii* can be cultured in brackish water (up to a salinity of 10‰), but with better production, individual size, and stock survival at a salinity of 5‰.

The variable pattern according to a trend of decreasing growth performance as salinity rose under the lower salinity range conditions than the isobaric salinity of the experimental prawn may relate to water accumulation during the molting of the prawns. Neufeld et al. 1993) commented that marine crustaceans often grow rapidly in water with low salinity

due to water absorption during molting. Blue crabs (*Callinectes sapidus*) living in water with low salinity always maintain osmotic blood concentrations much higher than in the external environment (Ballard & Abbott, 1969). The osmotic concentration difference between blood and water at low salinity causes blue crabs to absorb more water during molting and thus increase their body size (Defur et al., 1988). Neufeld et al. (1993) commented monitored the molting process in blue crabs, also finding that during the first hour after molting in water with low salinity (2‰) or high salinity (28‰), blue crabs absorb much water. This amount of water will contribute 71% to the increase in body mass when crabs are in low salinity water (2‰) and only 58% when crabs are in high salinity water (28‰). Several previous studies noted that black tiger shrimp (*Penaeus monodon*) in a low-salinity environment showed the same results. Rathacharen et al. (2005) experimented and found that when rearing black tiger shrimp in freshwater, the average weight of the shrimp was significantly larger than when cultured in seawater after 80 days of culture (p < 0.05). The research results of Diep (2012) also showed that although at a salinity of 3‰, black tiger shrimp use large amounts of energy for osmotic regulation, the average weight of shrimp at a salinity of 3‰ tends to be higher than at salinities of above 3‰. This result may be due to the influence of water infiltration into the body of shrimp in a hypotonic environment, especially during the molting period. Diep (2012) found that the average water content in shrimp meat cultured at a salinity of 3‰ was higher than the average water content in shrimp meat cultured in the higher salinity range of 15-35‰; however, the difference was not significant (p > 0.05).

Growth and survival rate are important parameters to evaluate success in aquaculture, and these parameters are closely related to the yield (Gjedrem & Rye, 2018; Thitamadee et al., 2016). Although the lower AMGFP survival rates were at lower salinities in the salinity range of 0-15‰, the faster growth in weight and length of the AMGFPs in the salinity range of 0–5‰ seems to have helped their higher yield at this salinity range (**Figure 1-2** and **Table 2**). From the findings, it was recommended that a salinity range of 0-5‰ is considered to be suitable for grow-out culture for AMGFP juveniles.

Growth and survival rates of AMGFP juveniles at various alkalinities

Total alkalinity is an essential variable in aquaculture water. Higher alkaline waters have higher levels of CO_3^{2-}, HCO_3^-, Ca^{2+}, and Mg^{2+} ions, resulting in a greater buffering capacity and a more stable pH (Boyd, 2016). Moreover, the ion contents of Ca^{2+} and Mg^{2+} play a vital role in the molting process of prawns because it helps prawns form a new shell and harden the shell after the molting process. When giant freshwater prawns are cultured in water with a guaranteed Ca^{2+} content, the molting cycle of prawns will be shortened; thus, the prawns will grow faster (Adhikari et al., 2007). In addition, Funge-Smith (1991) found that as for the trace elements, bromide is necessary for the metabolism of *M. rosenbergii* in the larval and postlarval stages, and strontium dictates the magnesium regulation in an adult during their molt cycle. Therefore, if the preparation of the culturing medium lacked some essential elements, then that could cause a poor growth rate. According to Boyd et al. (2016), low-alkalinity water is considered less suitable for the culture of fish and prawns. Individual species requirements and the magnitude of the concomitant increase in pH value primarily define the upper limit of alkalinity. Although levels of between 40 and 100 $mgCaCO_3\ L^{-1}$ are recommended for a successful farming operation of *M. rosenbergii* (Adhikari et al., 2007; New & Singholka, 1985), an alkalinity range of 100–150 $mgCaCO_3\ L^{-1}$ tends to spend less energy on osmoregulation, results in better growth (Adhikari et al., 2007). Adhikari et al. (2007) found that alkalinity levels of 205 $mgCaCO_3\ L^{-1}$ or above reduced the growth rate of *M. rosenbergii*.

In the present study, the survival rates of AMGFPs were very little affected by the alkalinity range of 80 to 160 $mgCaCO_3\ L^{-1}$ (**Figure 3**) for all their growth performance parameters in weight and length. However, the yield of AMGFPs was highest at an alkalinity of 160 $mgCaCO_3\ L^{-1}$ and significantly different from those at lower alkalinities after 90 days of culture (**Table 3-4** and **Figure 4**). As mentioned above, survival rates and growth performance are closely related to yield. While survival was less varied in the investigated

alkalinity range, the faster growth performance of AMGFPs at an alkalinity of 160 mgCaCO$_3$ L^{-1} appears to have resulted in a higher yield. Wurts (1993) mentioned that fish could lose calcium in the water when they are in low calcium waters, and then they must use energy and ions supplied by their food to re-absorb lost calcium salts. That could be the case for extremely low calcium waters where prawns could lose their calcium through the exuviae. This finding may support explaining the results in our study that during the first 60 days of culture, when AMGFPs were small, their need for minerals for the outer shell was not yet significantly different. Therefore, there were no significant differences in the growth parameters (MW and ML, DWG, SGR$_W$, DLG, and SGR$_L$) among the investigated alkalinity levels (p > 0.05). From the 60th day to the end of the experiment, AMGFP body size increased in the tanks, leading to an increased need for minerals. This need may have led to the highest yield at an alkalinity of 160 mgCaCO$_3$ L^{-1} because of the best response to the growth of AMGFPs. Based on the findings of these two experiments, the optimal environment has a salinity range of 0–5‰ and alkalinity of 160 mgCaCO$_3$ L^{-1} for the growth-out culture of AMGFP juveniles.

Conclusion

The salinity range of 0-15‰ and the alkalinity range of 80-160 mgCaCO$_3$ L^{-1} did not significantly affect the survival rate of AMGFP juveniles after the periods of growing out culture for 90 days. However, increased salinity significantly reduced the growth performance of the AMGFPs between salinities of 0, 5, 10, and 15‰, and the salinity range of 0-5‰ supported the highest prawn yield. In addition, the alkalinity range of 80-160 mgCaCO$_3$ L^{-1} did not significantly affect the growth performance of the AMGFPs in the first 60 days of the culture. However, the AMGFP growth performance and yield reached their highest values at an alkalinity of 160 mgCaCO$_3$ L^{-1}. Both were significantly reduced in the alkalinity range of 80-140 mgCaCO$_3$ L^{-1} after 90 days of culture. It is recommended that the salinity range of 0-5‰ and alkalinity of 160 mgCaCO$_3$ L^{-1} be applied for the grow-out culture of AMGFP juveniles. Additionally, upper alkalinities of 160 mgCaCO$_3$ L^{-1} need to be investigated to find the best alkalinity for the culture of AMGFP juveniles.

Acknowledgments

References

Adhikari, S., Chaurasia, V. S., Ahmad, A. N., & Pillai, B. R. (2007). Survival and growth of *Macrobrachium rosenbergii* (de Man) juvenile in relation to calcium hardness and bicarbonate alkalinity. *Turkish Journal of Fisheries and Aquatic Sciences*, 7, 23-26.

APHA. 1989. Standard methods for examination of water and wastewater, 17th Edition. American Public Health Association. American Water Works Association, Water Pollution Control Federation. Published by the American Public Health Association, Washington DC, USA.

Avnimelech, Y. (2012). Biofloc technology - A Practical Guide Book, 2nd ed. *The World Aquaculture Society, Baton Rouge, Louisiana, EUA.* 272pp

Ballard, B. S., & Abbott, W. (1969). Osmotic accommodation in *Callinectes apidus* Rathbun. *Comparative Biochemistry and Physiology*, *29*, 671-687. https://doi.org/10.1016/0010-406X(69)91620-X

Biesterfeld, S., Farmer, G., Russell, P., & Figueroa, L. (2003). Effect of alkalinity type and concentration on nitrifying biofilm activity. *Water environment research, 75*(3), 196-204. https://doi.org/10.2175/106143003X140971

Boyd, C. (2016). What causes alkalinity changes in aquaculture waters? Global Aquaculture Advocate. https://www.globalseafood.org/advocate/what-causes-alkalinity-changes-in-aquaculture-waters

Boyd, C. E. (1979). Water quality in warm water fish ponds. *Agricultural Experiment Station, Auburn University*. *Alabama, USA*. 359pp

Boyd, C. E., & Tucker, C. S. (1992). Water quality and pond-soil analyses for aquaculture. *Alabama Agriculture Experimental Station, Auburn University. Alabama, USA*. 183pp.

Boyd, C. E, & Tucker, C. S. (2016). Alkalinity and Hardness: Critical but elusive concepts in aquaculture. *Journal of the World Aquaculture Society, 47*(1), 6-41. https://doi.org/10.1111/jwas.12241

Boyd, C. E., & Zimmermann, S. (2000). Grow-out systems-water quality and soil management. In: New, M. B, & Valenti, W. C. (Eds). Freshwater prawn culture: the farming of *Macrobrachium rosenbergii*. *Blackwell Science*, 221-238. https://doi.org/10.1002/9780470999554.ch14

Brody, T., Cohen, D., Barnes, A., & Spector, A. (1980). Yield characteristics of the prawn *Macrobrachium rosenbergii* in temperate zone aquaculture. *Aquaculture*, *21*, 375-385. https://doi.org/10.1016/0044-8486(80)90073-3

Chen, S., Ling, J., & Blancheton, J. P. (2006). Nitrification kinetics of biofilm as affected by water quality factors. *Aquacultural engineering*, *34*, 179-197. https://doi.org/10.1016/j.aquaeng.2005.09.004

Cheng, W., Liu, C. H., Cheng, C. H., & Chen, J. C. (2003). Osmolality and ion balance in giant river prawn *Macrobrachium rosenbergii* subjected to changes in salinity: role of sex. *Aquaculture Research*, *34*, 555-560. https://doi.org/10.1046/j.1365-2109.2003.00853.x

Cohen, D., Sagi, A., Ra'anan, Z., & Zohar (1988). The production of *Macrobrachium rosenbergii* in monosex populations. III. Yield characteristics under intensive monoculture conditions in earthen ponds. *Israeli Journal of Aquaculture - Bamidge*h, *40*, 57-63.

Colt, J. (2006). Water quality requirements for reuse systems. *Aquacultural Engineering, 34,* 143-156. https://doi.org/10.1016/j.aquaeng.2005.08.011

Davassi, L. A. (2011). Survival and growth of the freshwater prawn *Macrobrachium rosenbergii* in relation to different nutrients composition. *Journal of Fisheries and Aquatic Science*, *6*(6), 649–654. https://doi.org/10.3923/jfas.2011.649.654

Defur, P. L., Nusbaumer, D., & Lewis, R. J. (1988). Physiological aspects of moulting in blue crabs in tidal fresh water. *Journal of Crustacean Biology*, *8*, 12-19. https://doi.org/10.2307/1548425

Diep, D. X. (2012). Effects of salinity and dissolved oxygen on physiological changes and growth of black tiger shrimp (*Penaeus monodon* Fabricius, 1798). Doctoral thesis. *Can Tho University, Vietnam*. 203pp

Edwards, H. A. (1982) Aedes aegypti: Energetics of osmoregulation. *Journal of Experimental Biology*, *101*, 135–141. https://doi.org/10.1242/jeb.101.1.135

Ern Andersen, R. (2009). Effects of salinity on oxygen consumption and hemolymph composition in the giant freshwater prawn (*Macrobrachium rosenbergii*). Master thesis. *Aarhus University, Denmark*. 41 pp

Eslami, S., Hoekstra, P., Minderhoud, P. S., Trung, N. N., Hoch, J. M., Sutanudjaja, E. H., Dung, D. D., Tho, T. Q., Voepel, H. E., Woillez, M. N., & van der Vegt, M. (2021). Projections of salt intrusion in a mega-delta under climatic and anthropogenic stressors. *Communications Earth & Environment*, *2*(1), 1-11. https://doi.org/10.1038/s43247-021-00208-5

FAO. (2019). FAO yearbook*.* Fishery and aquaculture statistics 2017. Rome: *FAO Fisheries and Aquaculture Department*.

FAO. (2022). The state of world Fisheries and aquaculture 2022. Towards Blue Transformation. Rome, FAO. 266 pp. https://doi.org/10.4060/cc0461en

Food and Agriculture Organization of the United Nations (FAO). 2022. FishStatJ-Software for Fishery Statistical Time Series (version 4.01.9). (Computer Program). http://www.fao.org/fishery/statistics/soft ware/fishstatj/en. Cited 30 Sep 2021.

Funge-Smith S., Taylor, A., Whitley, J., & Brown J. (1995). Osmotic and ionic regulation in the giant Malaysian fresh water prawn, *Macrobrachium rosenbergii* (de Man), with special reference to strontium and bromine. *Comparative Biochemistry and Physiology Part A: Physiology*, *110*, 357-365. https://doi.org/10.1016/0300-9629(94)00170-X

Funge-Smith, S. J. (1991). Ionic aspects of the physiology and biology of *Macrobrachium rosenbergii* (De Man) 1879. Ph.D. Thesis. *Institute of Aquaculture, University of Stirling. Scotland, UK*. 220 pp

Furtado, P. S., Poersch., L. H. & Wasielesky. W. (2015). The effect of different alkalinity levels on *Litopenaeus vannamei* reared with biofloc technology (BFT). *Aquaculture International*, *23*, 345–358. DOI: 10.1007/s10499-014-9819-x

Gjedrem, T., & Rye, M. (2018). Selection response in fish and shellfish: a review. *Aquaculture*, *10*(1), 68-179. https://doi.org/10.1111/raq.12154

Ha, B. T. L. (2014). Research on technological solutions for sex control of giant freshwater prawns (*Macrobrachium rosenbergii*). The scientific report of the government-level project under the program "Development and application of biotechnology in the fisheries sector to 2020". *The*

Research Institute for Aquaculture No. 2 (RIA2) under the Ministry of Agriculture and Rural Development of Vietnam*.

Habashy, M. M., & Hassan, M. M. (2011). Effects of temperature and salinity on growth and reproduction of the freshwater prawn, *Macrobrachium rosenbergii* (Crustacea-Decapoda) in Egypt*. International journal of environmental science and engineering (IJESE)*, *1*, 83-90.

Hines, A. H., Lipcius, R. N., & Haddon, A. M. (1987). Population dynamics and habitat partitioning by size, sex, and molt stage of blue crabs *Callinectes sapidus* in a sub-estuary of central Chesapeake Bay. *Marine Ecology Progress Series, 36,* 55-64. DOI: 10.3354/meps036055

Huong, D. T. T., Wang, T., Bayley, M., & Phuong N. T. (2010). Osmoregulation, growth and moulting cycles of the giant freshwater prawn (*Macrobrachium rosenbergii*) at different salinities. *Aquaculture Research, 41*, 135-143. https://doi.org/10.1111/j.1365-2109.2010.02486.x

Huong, H. K., Son, L. P., Hai, T. N., & Huong, Đ. T. T. (2015). The effect of salinities on molting cycle, spawning and growth of giant freshwater prawn (*Macrobrachium rosenbergii*). *Scientific Journal of Can Tho University, 38,* 35-43.

Lemos, D., Phan, V., & Alvarez, G. (2001). Growth, oxygen consumption, ammonia-N excretion, biochemical composition and energy content of *Farfantepenaeus paulensis* Pérez-Farfante (Crustacea, Decapoda, Penaeidae) early postlarvae in different salinities. *Journal of experimental marine biology and ecology, 261,* 55-74. doi: 10.1016/s0022-0981(01)00260-x.

Levy, T., Rosen, O., Eilam, B., Azulay, D., Zohar, I., Aflalo, E. D., Benet A., Naor, A., Shechter, A., & Sagi, A. (2017). All-female monosex culture in the freshwater prawn *Macrobrachium rosenbergii* - A comparative large-scale field study. *Aquaculture, 479,* 857-862. https://doi.org/10.1016/j.aquaculture.2017.07.039

Lezer, Y., Aflalo, E. D., Manor, R., Sharabi, O., Abilevich, L. K., & Sagi, A. (2015). On the safety of RNAi usage in aquaculture: The case of all-male prawn stocks generated through manipulation of the insulin-like androgenic gland hormone. *Aquaculture, 435,* 157–166. https://doi.org/10.1016/j.aquaculture.2014.09.040

Libes, S. M. (1992). An introduction to marine biogeochemistry. *John Wiley & Sons Inc*, 36-37.

Lockwood, A. J. B. R. (1962). The osmoregulation of crustacea. *Biological Reviews, 37,* 257-305. https://doi.org/10.1111/j.1469-185X.1962.tb01613.x

Malecha, S. R. (1983). Commercial pond production of the freshwater prawn, *Macrobrachium rosenbergii* in Hawaii. In McVey, J. P. ed. CRC handbook of mariculture, Vol. 1, Crustacean aquaculture. *CRC Press, Boca Raton, Florida, 1,* 205-230.

Malecha, S. R., A.Nevin, P., Ha, P., Barck, L. E., Lamadrid-Rose, Y., Masuno, S. & Hedgecock, D. (1992). Sex-ratios and sex-determination in progeny from crosses of surgically sex-reversed freshwater prawns, *Macrobrachium rosenbergii. Aquaculture*, *105*(3-4)*,* 201–218. https://doi.org/10.1016/0044-8486(92)90087-2

Mallasen, M., Valenti, W. C., & Ismael, D. (2003). Effects of Nitrate Concentration on Larval Development of the Giant River Prawn, *Macrobrachium rosenbergii*. *Journal of Applied Aquaculture, 14*(3)*,* 55-69. https://doi.org/10.1300/J028v14n03_05.

Nair, M. C., Salin, K. R., Raju, M. S., & Sebastian, M. (2006). Economic analysis of monosex culture of giant freshwater prawn (*Macrobrachium rosenbergii* de Man): a case study. *Aquaculture Research, 37*(9), 949–954. https://doi.org/10.1111/j.1365-2109.2006.01521.x

Nair, C. M., & Salin, K. (2012). Current status and prospects of farming the giant river prawn *Macrobrachium rosenbergii* (De Man) and the monsoon river prawn M acrobrachium malcolmsonii (HM E dwards) in India. *Aquaculture Research, 43,* 999-1014.

Neufeld, D. S., & Cameron, A. N. (1993). Mechanism of the net uptake of water in moulting blue crabs (*Callinectes sapidus*) acclimated to high and low salinities. *Journal of Experimental Biology, 188* (1)*,* 11–23. https://doi.org/10.1242/jeb.188.1.11

New, M. B. (1995). Status of freshwater prawn farming: a review. *Aquaculture Research, 26*(1)*,* 1-54. https://doi.org/10.1111/j.1365-2109.1995.tb00859.x

New, M. B. (2002). Farming freshwater prawns: a manual for the culture of the giant river prawn (*Macrobrachium rosenbergii*). *Food and agriculture organization of the United Nations, Rome, Italy*. 428pp.

New, M. B., & Nair, C. M. (2012). Global scale of freshwater prawn farming. *Aquaculture Research*, *43*(7): 960-969. https://doi.org/10.1111/j.1365-2109.2011.03008.x

New, M. B., & Valenti, W. C. (2000). Freshwater prawn culture: the farming of Macrobrachium rosenbergii. *Oxford: Blackwell Science, Oxford*. 443pp

New, M. B., & Singholka, S. (1985). Freshwater prawn farming. A Manual for the culture of *Macrobrachium rosenbergii*. *FAO fisheries technical paper 225 (Rev 1), Rome, Italy*. 118 pp

Nielsen, D. L., & Brock, M. A. (2009). Modified water regime and salinity as a consequence of climate change: prospects for wetlands of Southern Australia. *Climatic Change,* 95, 523-533. https://doi.org/10.1007/s10584-009-9564-8

Ohs, C. L., D'Abramo, L. R., & Kelly, A. M. (2006). Effect of dietary administration of 17 a - methyltestosterone on the sex ratio of postlarval freshwater prawn, *Macrobrachium rosenbergii*, during the nursery stage of culture. *Journal of World Aquaculture Society,* 37, 328–333. https://doi.org/10.1111/j.1749-7345.2006.00045.x

Paz, J. D. (1984). The effects of borderline alkalinity on nitrification in natural water systems. Ph.D. Dissertation, *Polytechnic Institute of New York, New York*. 125pp

Ra'Anan, Z., Sagi, A., Wax, Y., Karplus, I., Hulata, G., & Kuris A. (1991). Growth, size rank, and maturation of the freshwater prawn, *Macrobrachium rosenbergii*: analysis of marked prawns in an experimental population. *The Biological Bulletin, 181,* 379-386. doi: 10.2307/1542358.

Rathacharen, S., Laxminarayana, A., Venkatasami, O., & Codabaccus, B. (2005). Experimental studies on acclimatization of marine shrimps, *Penaeus monodon* and *Metapenaeus monoceros* to freshwater. 12p. http://hdl.handle.net/1834/1083

Rosas, C., Sanchez, A., Dıaz, E., Soto, L. A., Gaxiola, G., & Brito, R. (1996). Effect of dietary protein level on apparent heat increment and post-prandial nitrogen excretion of *Penaeus setiferus*, *P. schmitti*, *P. duorarum* and *P. notialis* postlarvae. *Journal of the World Aquaculture Society, 27,* 92-102. https://doi.org/10.1111/j.1749-7345.1996.tb00598.x

Sagi, A., Ra'anan, Z., Cohen, D., & Wax, Y. (1986). Production of *Macrobrachium rosenbergii* in monosex populations: yield characteristics under intensive monoculture conditions in cages. *Aquaculture, 51,* 265-275. https://doi.org/10.1016/0044-8486(86)90318-2

Sagi, A. & Cohen, D. (1990). Growth, maturation and progeny of sex-reversed *Macrobrachium rosenbergii* males. *World Aquaculture Report, 21,* 87–90.

Sang, H. M., & Fotedar, R. (2004). Growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn (*Penaeus latisulcatus* Kishinouye, 1896) reared at different salinities. *Aquaculture, 234,* 601-61. https://doi.org/10.1016/j.aquaculture.2004.01.008

Setiarto, A., Augusto Strüssmann, C., Takashima, F., Watanabe, S., & Yokota, M. (2004). Short-term responses of adult kuruma shrimp *Marsupenaeus japonicus* (Bate) to environmental salinity: osmotic regulation, oxygen consumption and ammonia excretion. *Aquaculture Research, 35,* 669-67. https://doi.org/10.1111/j.1365-2109.2004.01064.x

Siddiqui, A. Q., Al-Hafedh, Y. S., Al-Harbi, A. H., & Ali, S. A. (1997). Effects of stocking density and monosex culture of freshwater prawn Macrobrachium rosenbergii on growth and production in concrete tanks in Saudi Arabia. J*ournal of the World Aquaculture Society, 28,* 106-112. https://doi.org/10.1111/j.1749-7345.1997.tb00968.x

Silvia, G. J., Antonio, U. R. A., Francisco, V. O., & Georgina, H. W. (2004). Ammonia efflux rates and free amino acid levels in *Litopenaeus vannamei* postlarvae during sudden salinity changes. *Aquaculture*, *233,* 573-581. https://doi.org/10.1016/j.aquaculture.2003.09.050

Singh, T. (1980). The isosmotic concept in relation to the aquaculture of the giant prawn, *Macrobrachium rosenbergii*. *Aquaculture, 20,* 251-256. https://doi.org/10.1016/0044-8486(80)90115-5

Spaargaren, D. J. C. B. (1975). Energy relations in the ion regulation in three crustacean species. *Comparative Biochemistry and Physiology Part A: Physiology, 51*(3)*,* 543-548. https://doi.org/10.1016/0300-9629(75)90339-4

Stern, S., Borut, A., & Cohen, D. (1987). Osmotic and ionic regulation of the prawn *Macrobrachium rosenbergii* (De Man) adapted to varying salinities and ion concentrations. *Comparative Biochemistry and Physiology Part A: Physiology, 86,* 373-379. https://doi.org/10.1016/0300-9629(87)90345-8

Straus, D. L., Robinette, H .R., & Heinen, J. M. (1991). Toxicity of un-ionized ammonia and high pH to post-larval and juvenile freshwater shrimp *Macrobrachium rosenbergii. Journal of the World Aquaculture Society*, 22(2), 128-133. https://doi.org/10.1111/j.1749-7345.1991.tb00725.x

Thitamadee, S., Prachumwat, A., Srisala, J., Jaroenlak, P., Salachan, P. V., Sritunyalucksana, K., Flegel, T. W., & Itsathitphaisarn, O. J. A. (2016). Review of current disease threats for penaeid shrimp in Asia. *Aquaculture, 452,* 69-76. https://doi.org/10.1016/j.aquaculture.2015.10.028

Tidwell, J. H., D'Abramo, L. R., Coyle, S. D., Bright, L. A., Shultz, C., Kupchinsky, Z., Schwartz, P., & Upstrom, C. (2015). Production characteristics of all-male and mixed-sex giant river Prawns, *Macrobrachium rosenbergii*, grown in earthen ponds in Kentucky and Mississippi USA. *Journal of the World Aquaculture Society*, 46, 139-148. https://doi.org/10.1111/jwas.12174

Ventura, T., Manor, R., Aflalo, E. D., Weil, S., Raviv, S., Glazer, L., & Sagi, A. (2009). Temporal silencing of an androgenic gland-specific insulin-like gene affecting phenotypical gender differences and spermatogenesis. *Endocrinology, 150,* 1278-1286. https://doi.org/10.1210/en.2008-0906

Ventura, T., Manor, R., Aflalo, E. D., Weil, S., Rosen, O., & Sagi, A. (2012). Timing sexual differentiation: full functional sex reversal achieved through silencing of a single insulinlike gene in the prawn, *Macrobrachium rosenbergii*. *Biology of reproduction, 86*(3), 90, 1-6, https://doi.org/10.1095/biolreprod.111.097261

Wickins, J. F., & Daniel O. C. L. (2002). Crustacean farming: ranching and culture, 2nd edition. *Blackwell Science, Oxford, UK*. 464pp

Williams, W. (2001). Anthropogenic salinisation of inland waters. *Hydrobiologia, 466*(1)*,* 329-337. https://doi.org/10.1023/A:1014598509028

Woo, N. Y., & Kelly, S. P. (1995). Effects of salinity and nutritional status on growth and metabolism of *Spams sarba* in a closed seawater system. *Aquaculture, 135,* 229-238. https://doi.org/10.1016/0044-8486(95)01003-3

Wurts, W. A. (1993). Understanding water hardness. *World Aquaculture, 24* (1)*,* 18.

Yen, P. T., & Bart, A. N. (2008). Salinity effects on reproduction of giant freshwater prawn *Macrobrachium rosenbergii* (de Man). *Aquaculture, 280,* 124-128. https://doi.org/10.1016/j.aquaculture.2008.04.035