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ORIGINAL ARTICLE

Partial replacement of seawater with crude salt in giant freshwater prawn *Macrobrachium rosenbergii* hatchery operation

Md Lokman Ali[1] | Md Mahamudul Hasan Mredul[1] | Md Rabiul Islam Rubel[1] | Shib Nath Pattadar[1,2] | Subha Bhassu[3] | Sadia Sharmin[4,5] | Md Aminur Rahman[6] | Thi Kim Ahn Tran[7,8] | Md Rushna Alam[1,8]

[1]Department of Aquaculture, Patuakhali Science and Technology University, Dumki, Patuakhali, Bangladesh

[2]School of Environment and Natural Resources, The Ohio State University, Columbus, USA

[3]Division of Genetics and Molecular Biology, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia

[4]Department of Coastal Studies and Disaster Management, University of Barishal, Barishal, Bangladesh

[5]Laboratory of Fish Ecophysiology, Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh, Bangladesh

[6]Department of Public Health Engineering (DPHE), Zonal Laboratory, Jashore, Bangladesh

[7]School of Agriculture and Natural Resources, Vinh University, Vinh, Vietnam

[8]School of Environmental and Life Sciences, University of Newcastle, Callaghan, New South Wales, Australia

Correspondence
Md Rushna Alam, Department of Aquaculture, Patuakhali Science and Technology University, Dumki, Patuakhali 8602, Bangladesh.
Email: rushna_alam@pstu.ac.bd

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Abstract

The giant freshwater prawn *Macrobrachium rosenbergii* hatchery operators often face difficulties in seawater transportation. Thus, we aimed to test the locally available crude salt as a suitable alternative to seawater in hatchery operations. For this purpose, seven treatments with different combinations of seawater, brine and crude salt (viz. T_1 = 100% seawater, T_2 = 100% brine solution, T_3 = 100% crude salt, T_4 = 80% seawater and 20% crude salt, T_5 = 70% seawater and 30% crude salt, T_6 = 60% seawater and 40% crude salt and T_7 = 50% seawater and 50% crude salt) were tested in the rearing of *M. rosenbergii* larvae. Water quality parameters exhibited no significant differences, but elemental (Na, Mg, Ca, K, Zn, Cu, Cd and Pb) assemblage (via main effect PERMANOVA) varied across the treatments. Most of the water elements revealed a significant bivariate relationship with the post larvae (PL) production parameters, and this was also confirmed by multivariate RELATE analysis. Among the elements, BEST analysis and the DISTLM model showed that ionic imbalance, especially Na^+ concentration, is the main modulator in the hatchery production cycle, productivity and PL survivability. This proves that seawater is the best culture media for larvae rearing but up to 30% replacement by crude salt could be possible at 12 g/L water salinity for giant freshwater prawn PL production.

KEYWORDS
crude salt, freshwater prawn hatchery, ionic imbalance, post larvae

1 | INTRODUCTION

The giant freshwater prawn *Macrobrachium rosenbergii*, a species of *Macrobrachium* genus, is primarily a freshwater species but requires brackish water for its larvae survival (New et al., 2009). *M. rosenbergii* is a key economic species in Southeast Asia, but it is also produced in Israel, Africa and South and Central America (Maliwat et al., 2021). Because of the global distribution of these aquatic species and

rapidly improving commercial growing methods, the annual production of the giant freshwater prawn is expected to reach 10.48 million tons by 2025 (Tacon, 2020).

Dependent on the natural seed availability, the grow-out culture of *M. rosenbergii* in Bangladesh has developed only in coastal areas. Although some hatcheries are operating to produce seed in a controlled environment using diluted seawater or brine solution, this production rate is very irregular and unable to satisfy market demand (Ahmed & Diana, 2015). The export potential of prawn meat and its contribution to the national economy of Bangladesh, has resulted in the rapid expansion of this species' culture practice (at 10% each year) and is raising the demand for post larvae (PL; DoF, 2019). This means that the pressure on natural resources is increasing, and hatchery seed production needs to be emphasized to maintain the production cycle.

Larval development of *M. rosenbergii* occurs in natural estuaries that contain brackish water (10–15 g/L salinity; Kanaujia & Mohanty, 2001; Prasanti et al., 2011), despite the fact that they grow, mature, fertilize and even hatch in freshwater (Chand et al., 2015). Considering the species' behaviour, hatcheries may be located near the sea or in coastal locations and can also be run inland. To manage water quality, most hatcheries currently use a clean water system with either water replacement or recirculation and rely on new seawater rather than recirculated water (Paul & Vogl, 2011).

The transportation of seawater is often labour-intensive and expensive, so to reduce the cost in prawn hatcheries located far from the coastline and close to large consumer markets, hatcheries rely on salt pan brine (concentrated evaporate seawater) for dilution to 12 g/L salinity (Ali, 2019). Thus, the use of brine solution constitutes a cornerstone in prawn hatchery. This technology may reduce the cost of transportation, but not in a marginally profitable label. In addition, the collection and transportation of brine solution from the salt pan to inland regions far away from coastal areas are again problematic. Therefore, using alternative culture media suitable for larval development and meeting operation costs is paramount important. Some initial studies were performed to evaluate the possible use of different culture media (i.e., brine, salt stock solution and synthetic chemicals) for the seed production of *M. rosenbergii* (Ali & Sattar, 2012; Mishra, 2016; Tunsutapanich, 1980; Yambot & Vera Cruz, 1986). These studies, however, reported a low survival rate and low productivity. But, there is no in deep information about the ionic constitution of the different culture mediums available. Therefore, this study aimed to assess the effects of culture medium reconstituted from crude salt, a locally available source, and its different combinations with diluted seawater in the rearing of *M. rosenbergii* larvae. It is hypothesized that specific seawater and crude salt ratio could not create an ionic imbalance that has reversal effects on the growth, survival and osmoregulation of the prawn larvae.

In this context, we assume that crude sea salt could be used as replacement seawater in the *M. rosenbergii* hatchery operation. We have completed this experiment with the following objectives to determine the best combination of seawater with the crude salt solution for sustainable PL production.

- To test the suitability of crude sea salt in *M. rosenbergii* PL production in hatchery; and
- To examine which element, in terms of ionic balance, play a role in hatchery productivity in means of rearing cycle and survival of larvae.

2 | MATERIALS AND METHODS

2.1 | Experimental design

The experiment was carried out in the Kuakata prawn hatchery in the Patuakhali district of Bangladesh for 40 days to evaluate the suitability of crude salt substitution with seawater in larvae rearing of giant freshwater prawn, *M. rosenbergii*. Twenty-one cemented tanks were used for this experiment with a capacity of 3000 L. Seven treatments having different combinations of seawater, brine and crude salt were assessed in the rearing of *M. rosenbergii* larvae. Treatment 1 (T_1), Treatment 2 (T_2), Treatment 3 (T_3), Treatment 4 (T_4), Treatment 5 (T_5), Treatment 6 (T_6) and Treatment 7 (T_7) experimental tanks supplied with 100% seawater, 100% brine solution, 100% crude salt solution, 80% seawater and 20% crude salt, 70% seawater and 30% crude salt, 60% seawater and 40% crude salt and 50% seawater and 50% crude salt solution, respectively at 12 g/L water salinity (Table S1). Each treatment was assigned to a completely randomized experimental design with three replications to minimize the effects of the position of the tank inside the hatchery and of ambient weather conditions.

2.2 | Experimental set-up and husbandry

Seawater (35 g/L salinity) was collected from the Bay of Bengal, and brine (160 g/L salinity) and crude salt were collected from salt pans of Cox's Bazar District, Bangladesh. Crude salt was dissolved in groundwater (aerated). After that, seawater, brine, and crude salt solutions were kept in a different holding tank to settle down mud or garbage. After settling down, seawater, brine solution and crude salt solution were transferred into the water treatment tank through a filter to remove any solid materials from the water using a submersible pump. Then seawater, brine and crude salt solution were diluted to prepare 12 g/L brackish water, suitable salinity for freshwater larvae, by mixing with underground freshwater. Three types of 12 g/L brackish water prepared from seawater, brine solution and crude salt were collected into three water treatment tanks separately with a capacity of 30,000 L. The storage tanks were covered with a shed to minimize sun-driven evaporation. The brackish water was bleached with 60% chlorinated bleaching powder at a dose of 12 mg L^{-1}, and aeration was performed for 24 h to kill all types of disease-producing agents such as protozoan parasites, bacteria, virus, fungus and so on. After that, brackish water was treated with 12 mg L^{-1} sodium thiosulphate, and the air was blown for 1 day to remove the chlorine. Then, it was kept undisturbed for 1 day to allow the settling down of the

precipitates. The supernatant clear water was then collected for the rearing of larvae.

Female prawns bearing grey-coloured eggs were collected from the Payra river of Bangladesh and transferred to the hatchery using oxygenated plastic drums via a pick-up van. Before moving to the hatching tank, broods were restrained in a temporary holding tank and treated with 3 mg L^{-1} iodine solution for 15 min to eradicate any bacterial contamination. Then, broods were kept in the hatching tank (500 L) containing brackish water of 6 g/L salinity until fertilized eggs were released. Continuous aeration in the tank, feeding (twice a day with pellet feed), and shelter was provided to the broods to create a comfortable condition for releasing eggs and restricting cannibalism. After the eventual releasing and hatching of eggs (at night), the larvae were collected from the hatching tank using scope nets of 200 μm mesh size and a plastic bucket. Larvae were treated with a 2 mg L^{-1} iodine solution for 20 min to eradicate cross-contamination. Finally, 1-day-old larvae were stocked in 21 cemented experimental rearing tanks with a stocking density of 80 larvae per litre (Saritha & Kurup, 2011). Larvae were fed with *Artemia* nauplii and egg custard according to the feeding chart described in Tables S2 and S3. At 3-day intervals, 30% of the rearing tanks' water was changed to maintain the water quality. Water was top-up from previously prepared brackish water (12 g/L salinity) which was subject to prior 24 h aeration and water salinity adjustment.

The larvae were reared up to the PL stage, when they took the shape of adult prawns and started swimming, changing from backward to forward movements and changing from upside to normal position. Larval metamorphosis was monitored each day, in the morning by observing sampled (at least 10) larvae under the light microscope following different larval stages described by Wei et al. (2014). We also recorded the dead animals, days of first PL appearance, and days of 100% PL development (production cycle). At the end of the experiment, PLs were counted and the survival rate was calculated for each treatment as % Survival = (No. of harvested PL/No. of stocked larvae) × 100.

2.3 | Monitoring of water quality

Water quality parameters of the experimental cemented tanks were recorded daily throughout the study period. Physico-chemical parameters included water temperature (°C), dissolved oxygen (DO; mg L^{-1}), pH, total alkalinity (mg L^{-1} $CaCO_3$), total ammonia (mg L^{-1}), nitrite (mg L^{-1}) and salinity (g/L). The temperature (°C) of the experimental tank water was recorded with the help of a digital thermometer three times daily (at 7:00 am, 5:00 pm and 9:00 pm). Thermostats incorporated with the water heaters were used to maintain a water temperature between 28 and 32°C. DO, water salinity, and pH were measured twice a day at 9.00 am and 5.00 pm using a multimeter of WP 600 series (Oakton PCD 650, Eutech Instrument). At the same time (twice a day), alkalinity, total ammonia, and nitrite were recorded using a spectrophotometer (DR 6000; Alam et al., 2020).

2.4 | Measurement of elements in the experimental tank water

About 100 ml of water samples were collected in high-density polyethylene bottles (acidified with 1% nitric acid) in 3-day intervals, prior to 30% water replacement in the experimental tanks, until the end of the production cycle from each replicated tank and stored at −20°C. The collecting pooled water samples were transported to the toxicology laboratory, Bangladesh Fisheries Research Institute (BFRI), Mymensingh using an icebox and kept at 4°C prior to thawing until required to complete the analysis. The concentration of sodium (Na), magnesium (Mg), calcium (Ca), potassium (K), zinc (Zn), copper (Cu), cadmium (Cd) and lead (Pb) were determined using an atomic absorption spectrophotometer (AAS; Buck Scientific: 225ATS) following the methods of American Public Health Association (APHA, 2012).

A detail of the sampling and analytical procedures is explained by Rahman et al. (2021). Briefly, a calibration curve was made using working standard solutions from the different concentrations of the certified reference materials. Then, the response of the unknown metal ions of the water samples was calculated from the individual calibration curve. The measurements were carried out with flame atomization settings, where the Deuterium lamp served as a background correction. The wavelength of 217, 228.8, 213.9, 324.7, 589.6, 766.5, 285.5 and 422.7 nm (sourced from hollow cathode lamp) were selected for Pb, Cd, Zn, Cu, Na, K, Mg and Ca content respectively. The argon was used as a carrier gas for the determination of Pb, Cd, Zn and Cu, while air-acetylene was used (as a fuel gas) for the determination of Na, K and Mg. Both acetylene and nitrous oxide was employed for the analysis of Ca content. Mean and standard deviations were performed from the results of the three per sampling point. All analyses were performed in triplicate and the average values were recorded.

The detection limit (DL) of AAS was obtained for each metal from the three standard deviations of the blank responses. Thus, the DL of AAS for Pb, Cd, Zn, Cu, Na, k, Mg and Ca were determined to be 0.013, 0.0028, 0.0033, 0.0045, 0.001, 0.01, 0.01 and 0.012 mg L^{-1}, respectively, which seems that the AAS was good enough to determine the lower level of tested metals concentration.

All samples and solutions were prepared with analytical grade reagents and distilled water additionally purified by a Milli-Q system (Millipore), which results in ultra-pure water with a specific resistivity of 18.2 MΩcm at 25°C. Stock solutions with 1000 mg L^{-1} of Cd, Cu, Na, K, Mg, Ca, Pb and Zn were used. The working standard solutions were prepared by serial dilutions from stock solutions and acidified with 0.014 mol L^{-1} HNO_3 (67.5%). Metal-specific calibration curves were prepared with a blank (0.014 mol L^{-1} HNO_3 only) and 5 standards (acidified with 0.014 mol L^{-1} HNO_3) containing elements in the following range: Cd (0.15–0.75 mg L^{-1}), Cu (0.25–0.75 mg L^{-1}), Ca (0.250.75 mg L^{-1}), K (0.25–1.00 mg L^{-1}), Mg (0.25–1.0 mg L^{-1}), Na (0.25–1.0 mg L^{-1}), Pb (0.50–2.0 mg L^{-1}) and Zn (0.2–0.8 mg L^{-1}); all standards with 0.014 mol L^{-1} HNO_3. The final water content in the

blank and in the working standards after the addition of the aqueous standards and acid varied between 1.5% and 4.5% (v/v).

2.5 | Statistical analysis

All raw data were $Log_{10}(x+1)$ transformed to reduce variability among the data point before statistical analysis. This transformation is the first attempt to attain normality and homogeneity of variance. Normality and homoscedasticity of data were checked via q-q plot and Levene's test in spss v. 26. Univariate one-way analysis of variance (ANOVA) and bivariate correlation (Two-tailed Spearman) were performed in IBM SPSS Statistics (version 26). Multivariate statistical analyses, for example, PERMANOVA, RELATE, BEST, mMDS plot, DISTLM and dbRDA plot, were conducted using Euclidian distance-based dissimilarity matrix in PRIMER 7 equipped with PERMANOVA$^+$ add-on.

3 | RESULTS

3.1 | Water quality parameters during the experimental time

Water quality parameters (mean ± *SD*) of different treatments are presented in Table 1. No significant difference ($p < 0.05$) in temperature ($p = 0.97$), dissolved oxygen ($p = 0.72$), pH ($p = 0.29$), total alkalinity ($p = 0.16$), ammonia ($p = 0.76$), nitrite ($p = 0.34$) or salinity ($p = 0.23$) were observed among the treatments due to using of water from the same source. Throughout the investigation, the pH of the water varied from 8.15 ± 0.14 at T_5 to 8.55 ± 0.21 at T_2. The uppermost mean DO concentration (6.67 ± 0.24 mg L^{-1}) was recorded in T_6, and the lowest (6.19 ± 0.18 mg L^{-1}) was recorded in T_5. Treatment T_3 had the highest total alkalinity (176.89 ± 4.26 mg L^{-1}), whereas T_7 had the least (161.00 ± 5.01 mg L^{-1}). Temperature, ammonia and nitrite ranged between 29.75 ± 0.44 °C (T_3) and 30.61 ± 0.20 °C (T_6), 0.21 ± 0.02 mg L^{-1} (T_3) and 0.27 ± 0.04 mg L^{-1} (T_6) and 0.63 ± 0.05 mg L^{-1} (T_1) and 0.76 ± 0.07 mg L^{-1} (T_7) respectively.

3.2 | Elemental concentration differences among different treatments

The different elements concentrations (mean ± *SD*) of sampled water from different treatments at 12 g/L water salinity are shown in Table 2. Sodium (Na), magnesium (Mg), calcium (Ca) and potassium (K) concentrations ranged from 203.17 ± 0.0.33 mg L^{-1} (T_2) to 351.39 ± 0.55 mg L^{-1} (T_3), 14.27 ± 0.02 mg L^{-1} (T_3) to 76.54 ± 0.26 mg L^{-1} (T_1), 9.27 ± 0.10 mg L^{-1} (T_3) to 53.24 ± 0.18 mg L^{-1} (T_1) and 7.71 ± 0.35 mg L^{-1} (T_3) to 47.27 ± 0.43 mg L^{-1} (T_1) respectively. Heavy metals Zn, Cu, Cd and Pb were more or less similar among the treatment water samples with a mean range value of 0.04 to 0.05 mg L^{-1}, 0.02 to 0.03 mg L^{-1} and 0.04 mg L^{-1} and 0.18 to 0.20 mg L^{-1} respectively.

Multivariate analysis was conducted to determine the dissimilarities among the element's concentration assemblage via PERMANOVA among treatments (Figure 1; Table 3). Euclidian distance-based 2D multi-dimensional scaling plots presented seven treatments, which were divided into four groups. Treatment T_3 exhibited greater distance in terms of elemental assemblage difference from the rest of the treatments considered in group A; T_6 and T_7 mostly aggregated together, were in group B. Similarly, T_5 and T_4 were in group C and T_2 and T_1 were found in another grouping as D. The main effect PERMANOVA analysis confirmed a significant difference (pseudo-$F = 14{,}261$, $p = 0.001$) in element concentration assemblage across the seven treatment groups when compared, and T_3 had the lowest concentration of elemental assemblage (Table 3). Surprisingly, a lack of significant difference was observed between treatments in pairwise post hoc test, although it was marginal (see Table S4), such as between T_6 and T_7 ($p = 0.079$), T_1 and T_2 ($p = 0.089$) and T_3 and T_5 ($p = 0.078$).

3.3 | Production efficiency of *M. rosenbergii* larvae under different combinations of seawater, brine solution and crude salt treatments

While comparing the rearing time (Figure 2a), one-way ANOVA showed six groups with significant differences between each

TABLE 1 Water quality parameters were measured during the experiment. The mean value of each parameter is presented with standard deviation as mean ± *SD*. One-way ANOVA analysis showed differences among the treatments ($n = 3$).

	Temperature (°C)	DO (mg L^{-1})	pH	Alkalinity	Ammonia (mg L^{-1})	Nitrite (mg L^{-1})	Salinity (g/L)
T_1	30.17 ± 0.80	6.56 ± 0.09	8.18 ± 0.22	167.00 ± 4.29	0.23 ± 0.03	0.63 ± 0.05	12.24 ± 0.14
T_2	30.42 ± 0.99	6.47 ± 0.19	8.55 ± 0.21	165.00 ± 5.09	0.27 ± 0.06	0.68 ± 0.07	12.23 ± 0.11
T_3	29.75 ± 0.44	6.26 ± 0.68	8.40 ± 0.20	176.89 ± 4.26	0.21 ± 0.02	0.67 ± 0.04	12.87 ± 0.10
T_4	30.12 ± 0.90	6.34 ± 0.09	8.19 ± 0.13	165.00 ± 5.83	0.23 ± 0.02	0.67 ± 0.07	12.94 ± 0.30
T_5	30.13 ± 0.1.03	6.19 ± 0.18	8.15 ± 0.14	164.00 ± 3.67	0.25 ± 0.02	0.73 ± 0.02	12.14 ± 0.35
T_6	30.61 ± 0.2	6.67 ± 0.24	8.35 ± 0.19	166.00 ± 7.24	0.27 ± 0.04	0.67 ± 0.05	12.54 ± 0.73
T_7	30.45 ± 1.03	6.37 ± 0.23	8.45 ± 0.20	161.00 ± 5.01	0.26 ± 0.07	0.76 ± 0.07	12.34 ± 0.37
F	0.205	0.610	1.372	1.859	0.553	1.248	1.558
p	0.969	0.719	0.292	0.159	0.760	0.341	0.231

TABLE 2 Elemental concentration (mean ± *SD* mg L^{-1}) was measured in the water samples collected from different treatments ($n = 3$).

	Na	Mg	Ca	K	Zn	Cu	Cd	Pb
T_1	214.43 ± 0.44	76.54 ± 0.26	53.24 ± 0.18	47.27 ± 0.43	0.04 ± 0.02	0.03 ± 0.00	0.04 ± 0.00	0.19 ± 0.10
T_2	203.17 ± 0.33	72.27 ± 0.44	52.67 ± 0.23	46.23 ± 0.34	0.04± 0.00	0.02 ± 0.00	0.04 ± 0.00	0.18 ± 0.10
T_3	351.39 ± 0.55	14.27 ± 0.02	9.27 ± 0.10	7.71 ± 0.35	0.05 ± 0.00	0.01 ± 0.00	0.04 ± 0.00	0.20 ± 0.11
T_4	241.82 ± 0.46	64.09 ± 0.21	44.45 ± 0.16	39.36 ± 0.35	0.04 ± 0.02	0.03 ± 0.00	0.04 ± 0.00	0.19 ± 0.10
T_5	255.52 ± 0.47	57.86 ± 0.19	40.05 ± 0.16	35.40 ± 0.40	0.04 ± 0.02	0.03 ± 0.00	0.04 ± 0.00	0.19 ± 0.10
T_6	269.21 ± 0.48	51.63 ± 0.14	35.65 ± 0.15	31.44 ± 0.40	0.05 ± 0.02	0.02 ± 0.00	0.04 ± 0.00	0.19 ± 0.10
T_7	282.91 ± 0.49	45.41 ± 0.14	31.26 ± 0.14	27.49 ± 0.39	0.05 ± 0.01	0.02 ± 0.00	0.04 ± 0.00	0.19 ± 0.10

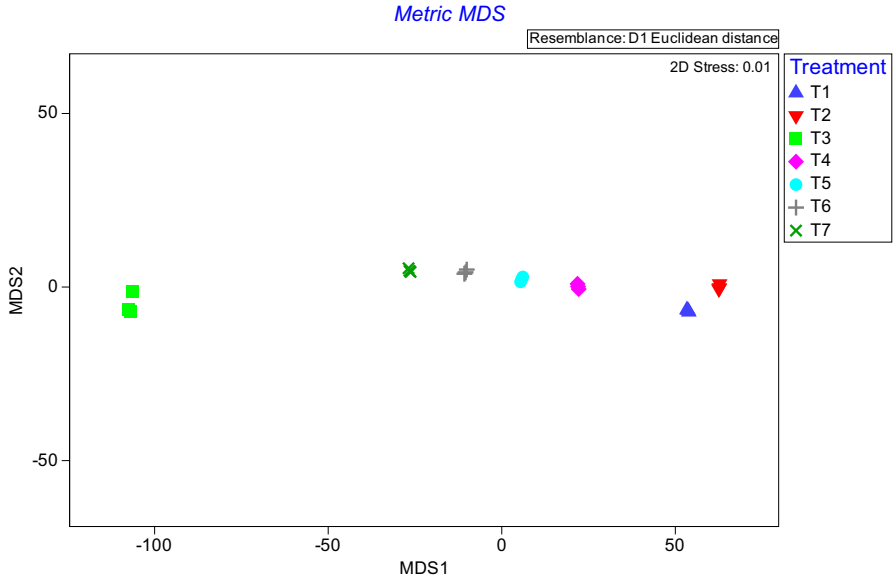

FIGURE 1 Two-dimensional metric multi-dimensional (mMDS) scaling ordination plot of the water samples element concentrations with different treatments rearing tanks supplied with different combinations of seawater, brine and crude salt solution based on Euclidean distance resemblance matrix.

TABLE 3 Main effect PERMANOVA table of the result of dissimilarities of elemental assemblage in the water samples among treatments based on Euclidian distance resemblance matrix. The significant difference is highlighted in bold.

Source	df	SS	MS	Pseudo-*F*	*p*(perm)	Unique perms
Treatments	6	59,092	9848.6	14,261	**0.001**	999
Res	14	9.6682	0.69058			
Total	20	59,101				

other (Table S5, $F = 120.73$, $p = 0.00$). Treatment T_3 required the minimum rearing time (5 ± 1.2 days) because all the individuals died before the first sampling, meaning no ultimate end products were attained and T_7 required (40 ± 1.02 days) the maximum rearing time. The other four treatments required the following rearing times: T_1 (28 ± 1.06 days), T_2 (29 ± 1.75 days) and T_4 (30 ± 1.51 days), T_5 (33 ± 1.02 days) and T_6 (36 ± 2.16 days). The survival rate was also significantly different among the treatments (Figure 2b; Table S5, $F = 182.25$, $p = 0.00$). The lowest survival rate was observed in T_7 (15%). Almost 50% survival rate was observed in T_1 (50.92 ± 2.16), T_2 (49.75 ± 1.72), T_4 (49.58 ± 2.42) and T_5 (48.65 ± 2.49). In the case of hatchery productivity, all treatments exhibited significant variation (Figure 2c and Table S5, $F = 111.63$, $p = 0.00$). The lowest production was observed in T_7 (11.47 ± 1.45 PL/L) and T_6 (28.58 ± 2.53 PL/L) and these treatments also required the maximum rearing time. However, there was no significant difference observed between T_1 (40.73 ± 2.65 PL/L), T_2 (39.81 ± 2.13 PL/L), T_4 (39.67 ± 3.17 PL/L) and T_5 (38.92 ± 1.87 PL/L) in pairwise post hoc test (Figure 2c). In T_1 first PL was observed within a shorter period (21 days) while T_7 larvae were required a maximum time (33 days) to convert into PL where seawater percentage was minimum (Table S6).

We performed a bivariate correlation analysis which represented the actual relationship between production parameters and physicochemical properties of water (Table 4). The survival rate was significantly correlated with Na ($r = -0.91$, $p = 0.00$), Mg ($r = 0.93$, $p = 0.00$), Ca ($r = 0.92$, $p = 0.00$), K ($r = 0.93$, $p = 0.00$), Zn ($r = -0.09$, $p = 0.00$), Cu ($r = 0.84$, $p = 0.00$) and Cd ($r = 0.57$, $p = 0.01$), while there was no significant relationship observed with lead ($r = 0.05$, $p = 0.84$). Among the water quality parameters, total alkalinity ($r = -0.73$, $p = 0.00$), Na ($r = -0.55$, $p = 0.01$), Mg ($r = 0.58$, $p = 0.01$), Ca ($r = 0.58$, $p = 0.01$), K ($r = 0.59$, $p = 0.01$), Cu ($r = 0.64$, $p = 0.00$) and Cd ($r = 0.49$, $p = 0.03$) showed significant impact on

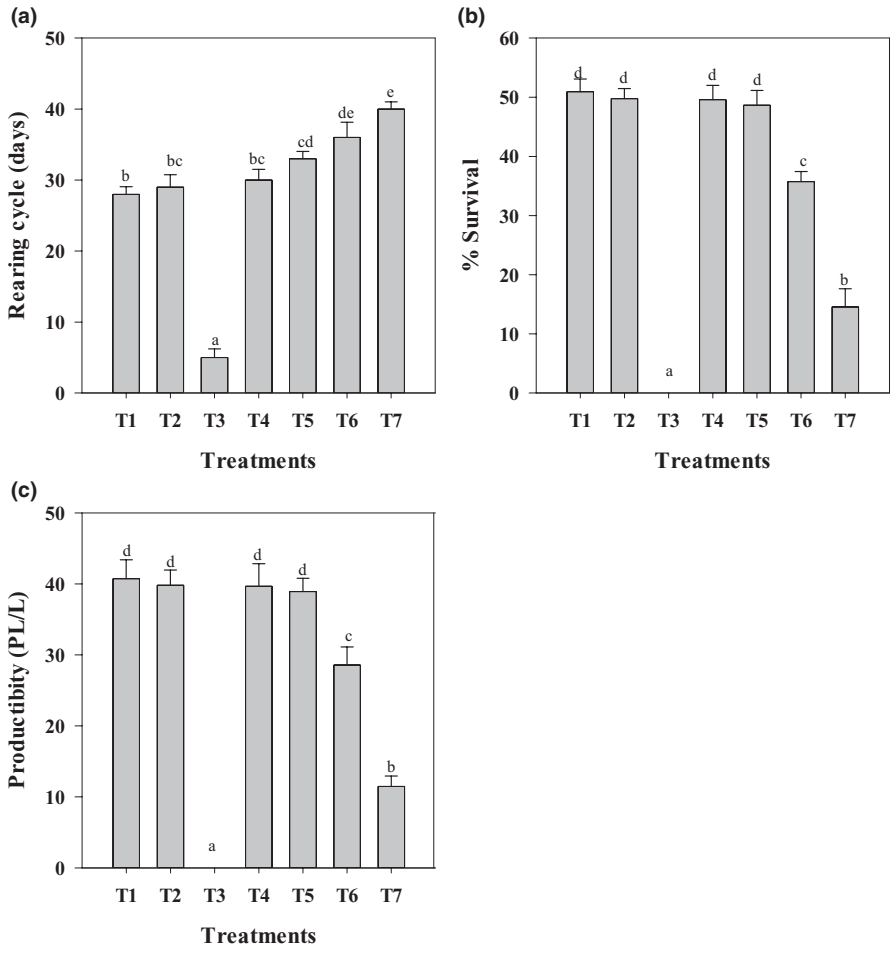

FIGURE 2 Production of *Macrobrachium rosenbergii* larvae reared in different treatments rearing tanks supplied with different combinations of seawater, brine and crude salt solution. Where (a) rearing cycle (days), (b) % survival and c. productivity (PL/L). ANOVAs examined differences among treatments of different combinations of seawater, brine and crude salt solution. Different letters indicate significant differences at $p < 0.01$ ($n = 03$).

rearing cycle. The concentration of elements such as Na ($r = -0.90$, $p = 0.00$), Mg ($r = 0.92$, $p = 0.00$), Ca ($r = 0.92$, $p = 0.01$), K ($r = 0.92$, $p = 0.00$), Cu ($r = 0.84$, $p = 0.00$) and Cd ($r = 0.59$, $p = 0.00$) showed significant correlation with hatchery productivity (Table 4).

To support bivariate relationships, we also ran a multivariate RELATE analysis based on the Euclidian resemblance dissimilarity matrix. RELATE analysis exhibited a significant relationship between *M. rosenbergii* larvae production parameters with experimental tank water physicochemical parameters and elemental concentration (Figure S1, Rho = 0.795 and significance level of sample statistic: 0.1%). In addition, the best analysis confirmed among the whole variable Na^+ concentration showed maximum correlation (0.994) with the production parameter of PL. The distance-based linear model (distLM) and db-RDA plot also showed that physicochemical properties of experimental tank water were the most important predictor for the production of *M. rosenbergii* PL (Figure 3). The result from db-RDA indicated T_3 was sharply separated from all the treatments, and lower production parameters were correlated with greater elemental concentration and water alkalinity. Collectively, the relationship showed that the survival rate, rearing time and productivity of larvae in T_1, T_2, T_4 and T_5 are not significantly different, but those are significantly different from T_6 and T_7. This variation occurred due to variation in the mineral concentration among seawater, brine and crude salt. This result is suggesting 30% of replacement of seawater with crude salt (T_5) could be attainable without any negative consequences on the productivity of the hatchery.

4 | DISCUSSION

Water quality plays an important role in freshwater prawn PL survival and production therefore, it is of the deepest concern for the researcher. In this experiment, the water quality parameters demonstrated no significant difference between treatments, but bivariate analysis indicates a significant correlation of water alkalinity with the rearing cycle. The growth of crustacean species is only possible by shedding and moulting of exoskeleton (Chang & Mykles, 2011; Gao et al., 2015). The concentration of cations (calcium–magnesium) required for the mineralization of the exoskeleton is represented by the alkalinity of the water; therefore, a small variation of alkalinity causes premature moulting and mortality of PL (González-Vera & Brown, 2017). Because moulting consumes a lot of energy and the newly fragile exoskeleton puts crustaceans at risk of death. Mineral reserves in tissues are often a good indicator of an animal's mineral health. Definitely, aquatic species might acquire part or all of their mineral demands from the water (Davis & Gatlin III, 1996). Previous research also confirmed that alkalinity correlated with the survival and production of the *M. rosenbergii* larvae, with higher

TABLE 4 Bivariate relationship between *Macrobrachium rosenbergii* larvae production parameters and water physicochemical properties (water quality parameters and elements in water sample) in rearing tank water.

		Temperature	DO	pH	Alkalinity	Ammonia	Nitrate	Salinity	Sodium (Na)	Magnesium (Mg)	Calcium (Ca)	Potassium (K)	Zinc (Zn)	Copper (Cu)	Cadmium (Cd)	Lead (Pb)
Rearing Cycle (days)	r	0.13	0.07	−0.14	−0.73	0.23	0.18	−0.41	−0.55	0.58	0.58	0.59	−0.02	0.64	0.49	0.11
	p	0.58	0.77	0.54	**0.00**	0.33	0.49	0.06	**0.01**	**0.00**	**0.01**	**0.01**	0.97	**0.00**	**0.03**	0.65
Productivity (PL/L)	r	−0.04	0.06	−0.37	−0.44	0.02	−0.28	−0.35	−0.90	0.92	0.92	0.92	−0.06	0.84	0.59	0.06
	p	0.87	0.80	0.09	0.05	0.93	0.22	0.12	**0.00**	**0.00**	**0.00**	**0.00**	0.80	**0.00**	**0.00**	0.75
% Survival	r	−0.01	0.07	−0.35	−0.42	0.04	−0.26	−0.33	−0.91	0.93	0.92	0.93	−0.09	0.84	0.57	0.05
	p	0.96	0.77	0.12	0.06	0.87	0.26	0.15	**0.00**	**0.00**	**0.00**	**0.00**	0.69	**0.00**	**0.01**	0.84

than 200 mg L^{-1} $CaCO_3$ considered lethal for PL (González-Vera & Brown, 2017).

M. rosenbergii has hyper-osmoregulatory abilities in fresh water and at low salinities, and the gill epithelium is thought to play a pivotal role in maintaining hemolymph NaCl balance in this process (Faleiros et al., 2017; Wilder et al., 2009). Because they lack the same osmoregulatory abilities, *M. rosenbergii* larvae cannot survive in freshwater for more than 24 h after hatching (Huong et al., 2010). It is worth noting that the concentrations of sodium, calcium, magnesium and potassium salts vary a lot among the various global aquatic environments where *M. rosenbergii* is cultivated. Our study results showed Na, Mg, Ca, K, Zn and Cu had a significant relationship with prawn larvae survival. Among them, Na^+ concentration is highly correlated (influenced) with this larvae production parameters. These comprises both macro and micro-elements, most of which are ionic in water, those are also essential for the organism's numerous biological and physiological functions.

The role of environmental salinity and Na^+ concentration was observed by Rafiee et al. (2015), and they concluded in their findings that there was a considerable drop in hemolymph protein content and an increase in the concentration of free amino acid in muscle tissue when an adult *M. rosenbergii* shifted from diluted concentration to more strenuous medium. These results indicate how Na^+ concentration affects the survival and production of *M. rosenbergii* PL. The proportional amounts of Na, Ca and Mg exposed in *M. rosenbergii's* aquatic habitat would fluctuate their respective concentrations in the hemolymph due to osmoregulation and moulting activities.

Several studies also agreed with our study result as they demonstrated that Na, Mg and K concentrations play a significant role in survival, moulting (Wilder et al., 2009), frequency and hemolymph osmolarity (Adhikari et al., 2007; Rezaei Tavabe et al., 2015). Roy et al. (2007) detected a positive correlation between white shrimp PL survival following exposure to low salinity water and levels of K^+ concentration. Because of the balanced availability of trace metals in seawater, first, PL were noticed in T_1 (100% seawater) within a shorter period (21 days). Metamorphosis time increased when the percentage of seawater in culture media decreased, owing to a rise in the balance of trace elements in the water. Larvae in T_7 took the longest (33 days) to convert into PL. According to Rezaei Tavabe et al. (2015), the optimum performance for *M. rosenbergii* larvae culture was 150 mg L^{-1} potassium, which provided the maximum final survival to PL metamorphosis. The results showed that pure seawater is the optimum media for giant freshwater prawn PL production. To support maximum growth and survival, potassium and magnesium concentration should be managed in culture media at appropriate levels. Our result is completely in agreement with Shuhaimi-Othman et al. (2011) in terms of the relationship between Cu concentration with survival and production, as Cu is a functional part of respiratory protein hemocyanin. High concentrations of Zn and Pb act as osmoregulatory inhibitors in freshwater prawns PL, the lack of substantial influence of Zn and Pb suggests crude salt as a suitable option for larvae rearing (Liu et al., 2021). Cd is a non-essential and toxic heavy metal for both human and aquatic organisms, but our result demonstrates a significant relationship with

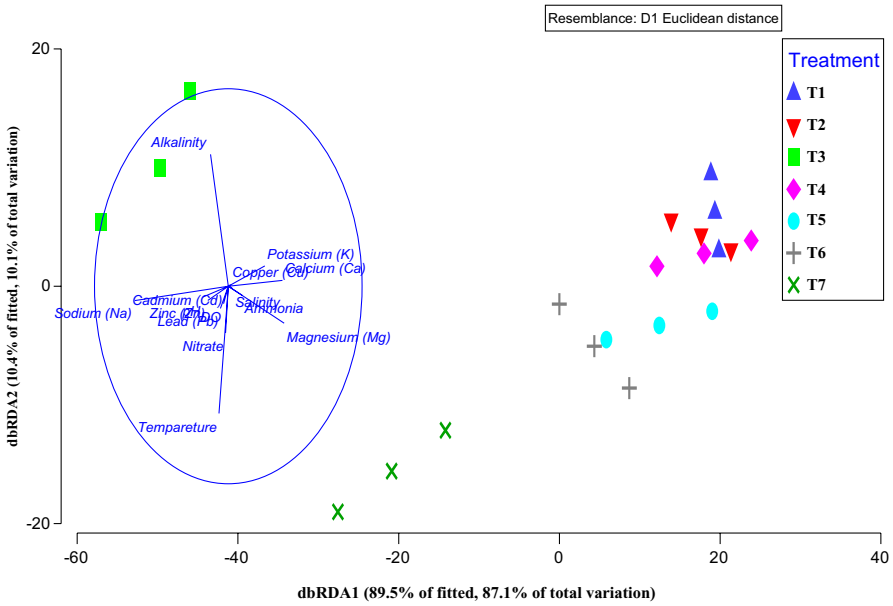

FIGURE 3 dbRDA ordination plot of DISTLM results of *Macrobrachium rosenbergii* post larvae production to water physiochemistry with vector overlay of production parameters.

the rearing cycle, survival rate and production, and so it deserves further deep assessment (Idrus et al., 2021).

This study also suggests that crude saltwater can replace up to 30% of seawater without affecting larvae survival. Miglio et al. (2021) concluded that the depletion of Mg^{2+} and K^{+} could cause high mortalities in the PL stage. Brine water could be used with reduced PL productivity. According to Jain et al. (2005), artificial seawater mixed with all essential components can boost survival rates, but it takes 5–10 days longer to produce since more energy is expended to maintain ionic equilibrium. So, it was obvious that when seawater or brine is replaced with crude salt at a rate of 40% or more, prawn larvae production would be possible and may give higher survival rate than this study. However, the variance in larval survival was discovered to be attributable to variations in trace metal concentrations in saltwater, brine and crude salt, according to this study.

5 | CONCLUSIONS

The present experiment assessed the suitability of crude salt in the freshwater giant prawn hatchery operation regarding water quality, ionic balance of culture medium and hatchery productivity. The temperature, DO, pH, alkalinity, nitrite and water salinity remained similar among the treatments during the experiment ($p > 0.05$). But, elemental assemblages were significantly varied across the seven treatments (main effect PERMANOVA, $p < 0.05$), where T_3 treatments showed the highest distance (highest elements assemblage concentration) from the other treatments. Furthermore, in terms of PL production efficiency, rearing time (days), survival rate (%) and production (PL/L) were significantly different among the treatments ($p < 0.05$). Collectively, T_1 showed the best performance with the shortest rearing time (28 ± 1.06 days), highest survival rate (50.92 ± 2.16%) and PL production (40.73 ± 2.65 PL/L); though these production parameters were very close to those of treatments T_2, T_4 and T_5. The majority of elements in the culture medium showed a significant relationship with production parameters, where BEST analysis and DISTLM model exhibited Na^{+} was the main modulator in giant freshwater PL hatchery operation.

The overall results of the study indicate that seawater is the best media for the production of giant freshwater prawn larvae. In the case of inland hatcheries far from the sea, brine could be effectively applied in rearing *M. rosenbergii* larvae. In any shortage of seawater or brine, crude salt can be replaced up to 30% without hampering significant production. It could also reduce the production cost, although this requires further investigation. Replacement of seawater or brine with crude salt above 40% is not viable for the commercial aspect of hatchery production. Crude salt is not the proper medium for larvae rearing of *M. rosenbergii* because there is an imbalanced concentration of elements present in crude salt compared with seawater.

In future research work, it is highly recommended to do characterization of elements concentration in seawater, brine salt and groundwater before preparing the culture medium by mixing them to explore further insight into ionic balance and to visualize the effect of ionic concentration ratios on the *M. rosenbergii* PL production parameters.

AUTHOR CONTRIBUTIONS

Md Lokman Ali: Conceptualization; methodology; investigation, data curation; draft writing; **Md Mahamudul Hasan Mredul**: Draft writing; review & editing; **Md Rabiul Islam Rubel:** Draft writing; review & editing, **Shib Nath Pattadar:** Validation; and review & editing, **Subha Bhassu**: Validation; and review & editing; **Sadia Sharmin:** Validation; writing, review & editing, **Md Aminul Rahman:** Validation; and review & editing, **Thi Kim Ahn Tran**: Validation; and review & editing, **Md Rushna Alam**: Conceptualization; data analysis; validation; resources; draft writing, review & editing and administration.

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CONFLICT OF INTEREST

The authors declared that nothing to disclose that could affect the publication.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Subha Bhassu https://orcid.org/0000-0001-8658-6900

Md Rushna Alam https://orcid.org/0000-0002-3757-8928

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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