Interactions of different sodium and potassium concentrations on *Macrobrachium rosenbergii* (de Man) offspring quality parameters

Kamran Rezaei Tavabe¹, Gholamreza Rafiee¹, Michael Frinsko² & Harry Daniels³

¹Fisheries Department, Natural Resources Faculty, University of Tehran, Karaj, Iran
²North Carolina Cooperative Extension Service, North Carolina State University, Trenton, NC, USA
³Department of Biology, North Carolina State University, Raleigh, NC, USA

Correspondence: K R Tavabe, Fisheries Department, Natural Resources Faculty, University of Tehran, Karaj, Iran.
E-mail: krtavabe@ut.ac.ir

Abstract

The present study evaluated various sodium and potassium concentrations in hatchery water to determine which proportions would be optimal for *Macrobrachium rosenbergii* larviculture. Using a closed RAS system (60-L), experiments were conducted in two stages. In the first stage, larval quality parameters were compared among triplicate treatments of sodium (2000, 3000, 4000 and 5000 mg L⁻¹) and potassium (100, 150, 200 and 250 mg L⁻¹). During the second stage, these same parameters were compared from interactions of the two best concentrations determined in the first stage. Initial larval density was fixed at 100 larvae L⁻¹ and larval quality parameters such as larval stage index (LSI), larval condition index (LCI), larvae dry weight, survival (%), LC₅₀-24 h for formalin stress and time of the first postlarvae (PL) appearance were measured. Results showed that during the early larval period time LSI, LCI and survival parameters were affected only by potassium and the interaction with sodium was not significant. At a later period of the larval development, interactions between both sodium and potassium were measurable for LSI (*P* < 0.05) while the interactions on LCI and survival were not significant. Measurable differences among the combined treatments 4000 mg L⁻¹ sodium and 150 mg L⁻¹ potassium resulted in the best performance for *M. rosenbergii* larviculture. This concentration also provided the highest final survival to PL, metamorphosis (40.6 ± 2.5%) which was at least 10% higher than the other treatments.

Keywords: *Macrobrachium rosenbergii*, larval condition index, larval stage index, sodium, potassium

Introduction

*Macrobrachium rosenbergii* is native throughout Southeast Asia. Due to its popularity and great aquaculture potential, it has been transplanted throughout many other similar regions, globally (Tidwell, D’Abramo, Coyle & Yasharian 2005; New & Nair 2012). This species thrives in tropical and subtropical freshwater environments with access to adjacent brackish water areas; larval development requires low concentrations of environmental salinity (John 1957; Sandifer, Hopkins & Smith 1975; Singh 1980). In nature, breeders migrate from rivers to an estuary for spawning. After hatching, the larvae remain in brackish water until metamorphosis to the postlarvae (PL) stage (Ling 1969; Uno & Kwon 1969). Soon after metamorphosis, juveniles migrate to upstream freshwaters for final growth and maturation to adults (Ling 1969; Sandifer & Smith 1985; New 2000, 2003).

*Macrobrachium rosenbergii* breeds easily and relatively frequently under hatchery conditions (Cavalli, Lavens & Sorgeloos 1999; Cavallo, Lavens & Sorgeloos 2001). To meet the brackish water requirements for larval production, most hatcheries mix locally available freshwater with seawater, commercial sea salt or commercial mineral supplements added to less expensive locally available salt sources (New 2003). The best larval production
results at salinity concentrations ranging from 12 to 16 ppt (New 2003). To minimize water/salt losses, stable water quality conditions and reduce production costs, recirculation hatchery systems have been developed for *M. rosenbergii* larviculture. Minimal water usage is very important for inland hatcheries as both transportation costs of natural seawater and the expense of using artificial sea-salt ingredients can both be quite high (Valenti, Daniels, New & Correia 2010). These inputs are operational expenses that impact final PL production costs and, therefore, commercial viability (Mallasen & Valenti 1998). As the freshwater prawn industry has expended, supplying cost effective and high quality brackish water has been one of the most significant challenges related to successful hatchery production (Funge-Smith, Taylor, Whitley & Brown 1995; Bart & Yen 2003; Phuong, Hai, Hien, Bui, Huong, Son, Morooka, Fukuda & Wilder 2006; Valenti et al. 2010). Use of artificial brackish water permits the location of hatcheries in areas distant from coastal saline resources, grow-out farms and consumer markets (Mallasen & Valenti 1998).

The ionic composition of the culture water is very important. For all crustaceans, it directly affects osmotic-dependant physiological activities, especially those pertaining to haemolymph (Greenaway 1993; Tavabe, Rafiee, Frinsko & Daniels 2013a). Extracellular sodium and potassium concentrations are affected by their availability in the culture environment (Gross 1959). Adults and larvae of the fresh water prawns are effective regulators of the major ions, when exposed to a wide salinity range (Stern, Borut & Cohen 1987; Funge-Smith et al. 1995; Wilder, Ikuta, Atmomarsono, Hatta & Komuro 1998). Sodium and potassium are also very important for *M. rosenbergii* larvae development. Their concentrations in hatchery water have been shown to affect haemolymph osmolality (Wilder et al. 1998), such that haemolymph sodium is hyper-regulated in salinities below the iso-ionic point (15 ppt), and in salinities above the iso-ionic point, the haemolymph sodium concentration follows that of the medium; potassium, however, is hyper-regulated at all salinities (Brown, New & Ismael 2010). Damrongphol, Jaroensastraraks and Poolsanguan (2001) indicated that the ionic requirement of newly hatched *M. rosenbergii* larvae differed from that of developing embryos. Although knowledge about larval osmoregulation of *M. rosenbergii* is of a great importance (Brown et al. 2010), few conclusive studies on the topic have been conducted, especially relative to the effects of water quality on larval rearing (Valenti et al. 2010).

In a previous study by our laboratory, we revealed that brackish water sources with the same salinity and different sodium and potassium concentrations showed different performance results (Tavabe, Rafiee, Frinsko & Daniels 2013b). We have also shown that different sodium adsorption ratio (SAR) mediums elicit different responses on larval quality (Rafiee, Tavabe, Frinsko & Daniels 2013). The question remained, ‘what is the best ratio of sodium and potassium in *M. rosenbergii* hatchery brackish water?’ New (2003) has mentioned that the specific ionic composition for this species larviculture is not known. Wilder et al. (1998) showed that in *M. rosenbergii*, changes in sodium haemolymph concentration generally paralleled changes in environmental osmolality, and potassium haemolymph concentration increased after exposure to extremely high salinity. In this latter case, their contents increased nearly twofold in response to changing salinity. Therefore, concentrations of these elements are very important in *M. rosenbergii* larviculture both independently as well as in combination. The main objectives of this study were to determine sodium and potassium interactions on growth-related larval quality parameters and to find the optimal balance of these important macroelements. The goal is to provide insight which can be used in additional studies to determine mechanisms of larval osmoregulation and also to improve hatchery success in those inland prawn hatcheries without a natural seawater supply. The resulting knowledge may assist the development of more efficient hatchery production of *M. rosenbergii*, enhancing future development of the freshwater prawn industry.

### Material and methods

A series of experiments were conducted in two stages at the aquaculture laboratory of the Fisheries Department of Natural Resources Faculty of Tehran University and at David Clark Laboratories in the Department of Biology at North Carolina State University. In the first stage, a comparison was made on the effects of various concentrations of sodium and potassium, separately, on freshwater prawn larval quality parameters. For the second stage, the two best concentrations of
macrolelements from the first stage were tested in combination. From these results, we then determined what macrolelement ratios could be recommended for larvae production.

Broodstock sources

The broodstock for the initial study \( (n = 76) \) were obtained from the Ghasreshirin freshwater prawn hatchery centre in Kermanshah province in western of Iran. Testing was conducted at the aquaculture laboratory of the Fisheries Department of Tehran University. Broodstock for the second experiment \( (n = 54) \) were obtained from a private farm in Kenly, North Carolina (USA) which were transported to the Lake Wheeler hatchery at North Carolina State University for testing. Both sources of the broodstock were of the same age \( (7 \pm 1 \text{ months}) \), similar individual weight and total length (Table 1). In the broodstock tanks, water quality parameters, photoperiod and feeding activity were monitored in accordance with recommendations for prawn broodstock (New 2003; Nhan, Wille, Hung & Sorgeloos 2010).

Larval collection and rearing systems

In the first stage of the study, larvae collection and counting were carried out where egg-bearing females with greyish egg masses were selected from the broodstock population and moved into larval hatching tanks (Menasveta & Piyatiratitivokul 1980). Upon hatching, larvae were removed and placed in a 50-L plastic container for counting. Total larvae from each hatch were calculated by taking the mean number from ten 100-mL beaker samples and multiplying this number by the volume of the container. Larvae collection for the second stage was conducted as described by New (2003) using a simple water recirculation system (RAS). This culture system consisted of a rectangular hatching tank (300-L) and two cylindrical tanks (120-L), one for collecting larvae and another one to house biofilter media. After the larvae were collected from the systems, they were moved to the larviculture treatment containers.

Three methods of M. rosenbergii larviculture have been suggested by Menasveta and Piyatiratitivokul (1980) for laboratory rearing. Because of the necessity to maintain a stable macrolelement concentration, the rearing systems were managed with minimum water exchange. Water quality parameters were adjusted following Nhan, Wille, Hung and Sorgeloos (2009). Total Ammonia-Nitrogen (TAN: NH₄-N) and Total Nitrite-Nitrogen (NO₂-N) were maintained below 0.2 and 0.1 mgL⁻¹ respectively. Gentle aeration was applied in all rearing containers. Average water temperature and dissolved oxygen were 30 ± 1°C and 7 ± 1 mgL⁻¹. A lamp system was installed, providing around 900–1000 lx for a 12 h day⁻¹ photoperiod.

The larvae were fed with Artemia franciscana (Great Salt Lake strain) nauplii twice daily at 7.00 am and 17.00 pm. Feeding densities, were adjusted as needed for each larval stage based on strategies developed by Barros and Valenti (2003a). Water quality was checked frequently and maintained to ensure a suitable culture environment. Overfeeding was avoided to reduce contamination by excess excretory wastes. In contrast, underfeeding was also monitored to avoid reduction of growth rate and the incidence of cannibalism (Valenti & Daniels 2000; Nhan et al. 2010).

Table 1 General and reproductive characteristics (mean ± SD) of both broodstock sources females

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ghasreshirin Hatchery (IR)</th>
<th>Lake Wheeler Hatchery (USA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (month)</td>
<td>7 ± 1</td>
<td>7 ± 1</td>
</tr>
<tr>
<td>Total length (cm)</td>
<td>15.8 ± 5.9</td>
<td>15.4 ± 4.6</td>
</tr>
<tr>
<td>Mean Weight (g)</td>
<td>35.2 ± 6.1</td>
<td>34.7 ± 5.3</td>
</tr>
<tr>
<td>Intermolt period (days)</td>
<td>31 ± 4</td>
<td>35 ± 5</td>
</tr>
<tr>
<td>Interspawn period (days)</td>
<td>45 ± 6</td>
<td>47 ± 5</td>
</tr>
<tr>
<td>Egg wet weight (µg)</td>
<td>89.2 ± 6.5</td>
<td>91.1 ± 5.4</td>
</tr>
<tr>
<td>Eggs per spawning (eggs female⁻¹)</td>
<td>42351 ± 6472</td>
<td>40850 ± 7346</td>
</tr>
<tr>
<td>Fecundity (egg g⁻¹ female)</td>
<td>1147 ± 408</td>
<td>1092 ± 517</td>
</tr>
<tr>
<td>Egg-clutch somatic index (ESI) (%)</td>
<td>9.6 ± 2.9</td>
<td>9.5 ± 2.5</td>
</tr>
</tbody>
</table>

Experimental set up and design

For the first stage, larvae were held in a closed RAS system (60-L) with biofilter. For the second stage, a static water system (10-L) was used. To maintain macrolelement concentrations in each container, partial water exchanges were conducted as needed, by siphoning. In both stages, initial larval density was fixed at 100 larvae L⁻¹ for each treatment. For each of the experiments, treatments were set up in triplicate. The composition of
artificial brackish water was created to mimic the salinity of brackish water sources freshwater prawn larvae thrive in (New 2003). Treatment concentrations for sodium and potassium were adjusted by varying the amount of their soluble salts, NaCl and KCl. For all experiments, initial potassium and sodium concentrations found in both laboratory waters were determined and their values incorporated when calculating treatment proportions. Ionic composition was confirmed by flame atomic spectrophotometry (Perkin Elmer AAnalyst 100 instrument; Perkin Elmer, Waltham, MA, USA).

Evaluation parameters

Broodstock reproductive parameters

Initial mean weight, total length, mortality, molting and duration of intermolt period of the females were recorded. Egg clusters were removed 7 days after spawning from berried females \( (n = 5) \) to estimate fecundity and egg wet weight. Egg-clutch somatic index (ESI) was calculated as an important female reproductive parameter.

\[
\text{Egg fecundity (eggs g}^{-1} \text{female)} = \frac{\text{Total eggs}}{\text{female weight}}.
\]

\[
\text{ESI} = \frac{\text{Egg clutch weight}}{\text{body weight}} \times 100
\]

Larval quality parameters

Larval dry weight, larval stage index (LSI), survival and time of the first PL appearance stage were assessed as larval quality parameters. Larval dry weights were determined at 1, 8 and 16 days post hatch (dph) in accordance with Nhan et al. (2010). Dry weight was obtained at 60°C for 48 h. Survival (%) was calculated at 10, 20 and 30 dph, and time of the first PL appearance was recorded in the treatments.

Larval stage index (LSI) (Uno & Kwon 1969) and larval condition index (LCI) (Tayamen & Brown 1999; Maciel & Valenti 2012) were determined at 3, 10, 15 and 20 dph.

\[
\text{LSI} = \sum \frac{S_i}{N}
\]

\[
\text{LCI} = \sum P \cdot \left(10N\right)^{-1}
\]

where: \( S_i \) is the stage of the larvae (i = 1–11). N is number of larvae examined. P is the score recorded for each larva.

Another evaluation of larval quality was conducted using the formalin stress test developed by Kitsawat and Chuchot (1991). On stages 4, 7 and 11 of the larval development period, three groups of 50 larvae were exposed to five increasing concentrations of formalin (50, 100, 150, 200 and 250 mg L\(^{-1}\)) for 24 h. Mortality after 24 h was calculated based on the mean lethal concentrations for 50% of the population (24 h-LC\(_{50}\)).

Statistical analysis

In the first stage, larval dry weight, survival, LSI, LCI, LC\(_{50}\) and time of the first PL appearance were analysed by analysis of variance (One-way ANOVA) and significant differences among the means were found \( (P < 0.05) \) by Duncan’s test in SPSS version 19 (IBM, New York, NY, USA). In the second stage, two-way ANOVA (General Linear Model- Univariate) was performed to evaluate the interactions of the two best concentrations of sodium and potassium (obtained from the first stage of the study) combined with each other, on the larviculture parameters analysed using the same software.

Results

Temperature, pH, dissolved oxygen (DO), ammonia-N and nitrite-N were maintained at 30 ± 1°C, 7–7.6, 7 ± 1 mg L\(^{-1}\), <0.2 mg L\(^{-1}\) and <0.1 mg L\(^{-1}\), respectively, during the research. Table 1 shows general characteristics (age, total length and weight) and reproductive properties such as intermolt period, interspawn period, egg wet weight, eggs per spawning, fecundity and ESI of broodstock females. There were no significant differences in general characteristics and reproductive properties between the broodstock sources.

Sodium effect

Larvae survival at 10 and 20 dph had no significant differences among the treatments but at 30 dph did show significant difference in the 2000 mg L\(^{-1}\) treatment \( (P < 0.05) \). Also, time of the first PL appearance was significantly \( (P < 0.05) \) longer at 2000 mg L\(^{-1}\) compare with treatments of 4000 and 5000 mg L\(^{-1}\) treatments (Table 2). Larvae dry weight showed no significant differences among the treatments at 1 and 8 dph but at 16 dph weights were significantly greater at the 4000 mg L\(^{-1}\) level \( (P < 0.05) \) (Fig. 1).
Larval stage index and LCI indices provided interesting results. Towards the final stages of the larval period, differences could be found among the treatments of 2000/3000 mg L\(^{-1}\)/C0\(^1\) and 4000/5000 mg L\(^{-1}\)/C0\(^1\). With both parameters, at 5 dph there were no significant differences among the treatments, but at 20 dph the treatments 4000 and 5000 mg L\(^{-1}\)/C0\(^1\) showed significantly greater amount than the treatments at 2000 and 3000 mg L\(^{-1}\) (Figs 2 and 3). The results of table 3 confirmed these results showing at the 11th larval stage, larvae in the treatments 4000 and 5000 mg L\(^{-1}\) showed significantly (\(P < 0.05\)) higher tolerance to formalin stress compare with the other treatments (Table 3).

**Potassium effect**

The effects of different potassium concentrations on larvae survival, dry weight, LSI and LCI were more distinctive than differences among various sodium concentrations. At 10 dph, there is no significant difference in survival among the treatments, but at 20 and 30 dph there were significant differences for the 150 and 200 mg L\(^{-1}\). With both parameters, at 5 dph

### Table 2 Survival (%) and time of the first PL appearance (mean ± SD) at different sodium concentrations. The comparison is intergroup and different letters at each row denote significant differences (\(P < 0.05\))

<table>
<thead>
<tr>
<th>Larval age (dph)</th>
<th>Sodium concentration (mg L(^{-1}))</th>
<th>2000</th>
<th>3000</th>
<th>4000</th>
<th>5000</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td>82 ± 6</td>
<td>81 ± 5</td>
<td>85 ± 9</td>
<td>83 ± 6</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>59 ± 8</td>
<td>63 ± 3</td>
<td>64 ± 4</td>
<td>66 ± 6</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>34 ± 4(\text{a})</td>
<td>41 ± 3(\text{a})</td>
<td>45 ± 5(\text{a})</td>
<td>42 ± 4(\text{a})</td>
</tr>
<tr>
<td>Time of the first PL appearance (day)</td>
<td></td>
<td>29 ± 1(\text{b})</td>
<td>27 ± 1.5(\text{ab})</td>
<td>26 ± 1(\text{a})</td>
<td>25 ± 1(\text{a})</td>
</tr>
</tbody>
</table>

**Figure 1** Larvae dry weight (mean ± SD) during development at different sodium concentrations. The comparison is intergroup and different letters at each group denote significant differences (\(P < 0.05\)). The groups that have not letters, their treatments did not show any significant differences.

**Figure 2** Larval stage index (LSI) (mean ± SD) during larval development at different sodium concentrations. The comparison is intergroup and different letters at each group denote significant differences (\(P < 0.05\)). The group that has not letters, its treatments did not show any significant differences.
treatments compared with the others. It is interesting that different potassium concentrations had no effect on time of the first PL appearance or larval period (Table 4). Figure 4 shows the dry weight of different potassium concentrations at different stages of the larval periods. At 1 dph, there is no significant difference among the treatments, but at 8 and 16 dph the treatments 150 and 200 mg L\(^{-1}\) showed significantly greater amounts than the other treatments (Fig. 4).

At 15 dph, LSI showed significantly (\(P < 0.05\)) greater differences in the treatments at 150 and 200 mg L\(^{-1}\) compared with 5 and 20 dph, which were significantly lower (\(P < 0.05\)) (Fig. 5). Interestingly, the LCI at 5 dph for the treatments 150 and 200 mg L\(^{-1}\) showed significant differences higher than the other treatments, but at 20 dph there were no significant differences among the treatments (Fig. 6). Results in the formalin stress test indicated higher tolerance in the 150 mg L\(^{-1}\) treatment at larval stages 4 and 7. At the 11th larval stage, the highest tolerance was similar in the 100, 150 and 200 mg L\(^{-1}\) treatments (Table 5).

Combined Na-K effects

The results show that the LSI treatment Na4000/K150 is significantly less at 5 dph than 10, 15 and 20 dph. However, the treatment Na5000/K200 is significantly greater than the others at 5 dph (\(P < 0.05\)). This further indicates that at 5 dph, potassium had an independent effect (\(P < 0.05\)) on LSI of without any interaction with sodium but at 10, 15 and 20 dph there was likely an interaction between the both sodium and potassium factors. Also, the time of the first PL appearance for Na4000/K150 treatment was significantly (\(P < 0.05\)) shorter. The results revealed that both sodium and potassium are affected independently and their interactions on the time of the first PL appearance were significant (Table 6).

The results also showed that there were no significant differences in larvae dry weight among

---

Table 3 The LC\(_{50}\)-24 h for formalin stress at various stages of larval development of *Macrobrachium rosenbergii* reared at different sodium concentrations. The comparison is intergroup and different letters at each column denote significant differences (\(P < 0.05\))

<table>
<thead>
<tr>
<th>Sodium concentrations (mg L(^{-1}))</th>
<th>LC(_{50})-24 h of formalin stress at different larval stages</th>
<th>Stage 4</th>
<th>Stage 7</th>
<th>Stage 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td></td>
<td>121 (\pm) 8(^b)</td>
<td>162 (\pm) 4(^b)</td>
<td>177 (\pm) 8(^a)</td>
</tr>
<tr>
<td>3000</td>
<td></td>
<td>124 (\pm) 9(^b)</td>
<td>164 (\pm) 6(^b)</td>
<td>179 (\pm) 5(^a)</td>
</tr>
<tr>
<td>4000</td>
<td></td>
<td>139 (\pm) 10(^a)</td>
<td>173 (\pm) 6(^a)</td>
<td>192 (\pm) 6(^a)</td>
</tr>
<tr>
<td>5000</td>
<td></td>
<td>134 (\pm) 9(^b)</td>
<td>171 (\pm) 5(^b)</td>
<td>190 (\pm) 5(^a)</td>
</tr>
</tbody>
</table>

---

Table 4 Survival (%) and time of the first PL appearance (mean \(\pm\) SD) at different potassium concentrations. The comparison is intergroup and different letters at each row denote significant differences (\(P < 0.05\))

<table>
<thead>
<tr>
<th>Larval ages (dph)</th>
<th>Potassium concentration (mg L(^{-1}))</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td></td>
<td>76 (\pm) 7</td>
<td>75 (\pm) 9</td>
<td>73 (\pm) 5</td>
<td>74 (\pm) 8</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>55 (\pm) 3(^b)</td>
<td>68 (\pm) 4(^a)</td>
<td>67 (\pm) 5(^a)</td>
<td>54 (\pm) 4(^b)</td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>22 (\pm) 4(^b)</td>
<td>35 (\pm) 6(^a)</td>
<td>36 (\pm) 5(^a)</td>
<td>19 (\pm) 7(^a)</td>
</tr>
<tr>
<td>Time of the first PL appearance (day)</td>
<td></td>
<td>28 (\pm) 2</td>
<td>28 (\pm) 3</td>
<td>29 (\pm) 2</td>
<td>30 (\pm) 3</td>
</tr>
</tbody>
</table>
the combined treatments. However, larvae survival showed significant differences \((P < 0.05)\) among the combined treatments (Table 7); at 10 dph for potassium, and 20 dph for sodium. Survival data at 30 dph indicate both factors had effects and interactions independent to each other (Table 7). LCI at 10 dph was significantly greater with the combined treatment Na4000/K150 (Table 8). Also, at 20 dph there were no significant differences among the treatments. Overall, this indicates, regarding LCI, there was interaction between the factors only at 10 dph. Other effects were independent and not significant (Table 8).
The results revealed that at different larval stages, *M. rosenbergii* larval quality parameters showed different responses to various concentrations of sodium and potassium separately and in combination. These results affirm that water salinity is important for larval quality, but also illustrate the significance of ionic and mineral composition to be even more important. At the first stage of the study, the treatments 4000 and 5000 mg L\(^{-1}\) sodium, 150 and 200 mg L\(^{-1}\) potassium indicated they were the best for larval growth and development. It is very interesting that at these early stages, the effects of the combination of sodium and potassium were more pronounced than their individual effects.

### Table 5

<table>
<thead>
<tr>
<th>Potassium concentrations</th>
<th>LC(_{50})-24 h of formalin stress at different larval stages</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stage 4</td>
</tr>
<tr>
<td>100</td>
<td>120 ± 7(^a)</td>
</tr>
<tr>
<td>150</td>
<td>138 ± 6(^a)</td>
</tr>
<tr>
<td>200</td>
<td>122 ± 5(^b)</td>
</tr>
<tr>
<td>250</td>
<td>118 ± 9(^b)</td>
</tr>
</tbody>
</table>

### Discussion

The results revealed that at different larval stages, *M. rosenbergii* larval quality parameters showed different responses to various concentrations of sodium and potassium separately and in combination. These results affirm that water salinity is important for larval quality, but also illustrate the significance of ionic and mineral composition to be even more important. At the first stage of the study, the treatments 4000 and 5000 mg L\(^{-1}\) sodium, 150 and 200 mg L\(^{-1}\) potassium indicated they were the best for larval growth and development. It is very interesting that at these early stages, the effects of the combination of sodium and potassium were more pronounced than their individual effects.

### Table 6

Larval stage index (LSI) at different dph and time of the first PL appearance (mean ± SD) of *Macrobrachium rosenbergii* larvae reared at different combined concentrations of sodium and potassium. The comparison is intergroup and different letters at each row denote significant differences (\(P < 0.05\)).

<table>
<thead>
<tr>
<th>Larval age (dph)</th>
<th>Combined treatments</th>
<th>Sodium factor ((P))</th>
<th>Potassium factor ((P))</th>
<th>Interaction ((P))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na4000/K150</td>
<td>Na4000/K200</td>
<td>Na5000/K150</td>
<td>Na5000/K200</td>
</tr>
<tr>
<td>5</td>
<td>3.8 ± 0.2(^a)</td>
<td>3.4 ± 0.3(^ab)</td>
<td>3.5 ± 0.2(^ab)</td>
<td>3.3 ± 0.3(^b)</td>
</tr>
<tr>
<td>10</td>
<td>6.6 ± 0.2(^a)</td>
<td>5.8 ± 0.4(^b)</td>
<td>5.9 ± 0.3(^b)</td>
<td>5.8 ± 0.2(^b)</td>
</tr>
<tr>
<td>15</td>
<td>8.1 ± 0.3(^a)</td>
<td>7.5 ± 0.2(^b)</td>
<td>7.5 ± 0.2(^b)</td>
<td>7.6 ± 0.2(^b)</td>
</tr>
<tr>
<td>20</td>
<td>10.3 ± 0.3(^a)</td>
<td>9.6 ± 0.4(^b)</td>
<td>9.5 ± 0.2(^b)</td>
<td>9.6 ± 0.3(^b)</td>
</tr>
<tr>
<td>Time of the first PL appearance</td>
<td>25.6 ± 0.6(^b)</td>
<td>30 ± 1.7(^b)</td>
<td>29.5 ± 1.5(^b)</td>
<td>29.6 ± 1.6(^b)</td>
</tr>
</tbody>
</table>

### Table 7

Larvae survival (mean ± SD) at different dph of *Macrobrachium rosenbergii* larvae reared at combined concentrations of sodium and potassium. The comparison is intergroup and different letters at each row denote significant differences (\(P < 0.05\)).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Larval age (dph)</th>
<th>Combined treatments</th>
<th>Sodium factor ((P))</th>
<th>Potassium factor ((P))</th>
<th>Interaction ((P))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae survival</td>
<td>10</td>
<td>74.6 ± 4.2(^a)</td>
<td>64.6 ± 3.2(^a)</td>
<td>72.3 ± 2.5(^a)</td>
<td>61.0 ± 4.1(^b)</td>
</tr>
<tr>
<td>(%)</td>
<td>20</td>
<td>49 ± 3.6(^a)</td>
<td>46.6 ± 4.1(^ab)</td>
<td>42 ± 4.4(^ab)</td>
<td>39.3 ± 3.8(^ab)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>40.6 ± 2.5(^a)</td>
<td>30.6 ± 3.2(^b)</td>
<td>29 ± 4.5(^b)</td>
<td>28.1 ± 5.5(^b)</td>
</tr>
</tbody>
</table>

### Table 8

Larval condition index (LCI) (mean ± SD) at different dph of *Macrobrachium rosenbergii* larvae reared at different combined concentrations of sodium and potassium. The comparison is intergroup and different letters at each row denote significant differences (\(P < 0.05\)).

<table>
<thead>
<tr>
<th>Larval age (dph)</th>
<th>Combined treatments</th>
<th>Sodium factor ((P))</th>
<th>Potassium factor ((P))</th>
<th>Interaction ((P))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Na4000/K150</td>
<td>Na4000/K200</td>
<td>Na5000/K150</td>
<td>Na5000/K200</td>
</tr>
<tr>
<td>5</td>
<td>1.84 ± 0.03(^a)</td>
<td>1.72 ± 0.06(^ab)</td>
<td>1.77 ± 0.11(^ab)</td>
<td>1.66 ± 0.04(^b)</td>
</tr>
<tr>
<td>10</td>
<td>1.83 ± 0.05(^a)</td>
<td>1.72 ± 0.04(^b)</td>
<td>1.71 ± 0.04(^b)</td>
<td>1.7 ± 0.03(^b)</td>
</tr>
<tr>
<td>15</td>
<td>1.81 ± 0.06(^a)</td>
<td>1.74 ± 0.08(^ab)</td>
<td>1.7 ± 0.09(^ab)</td>
<td>1.59 ± 0.08(^b)</td>
</tr>
<tr>
<td>20</td>
<td>1.69 ± 0.07</td>
<td>1.7 ± 0.08</td>
<td>1.63 ± 0.07</td>
<td>1.64 ± 0.09</td>
</tr>
</tbody>
</table>
larval stages, different concentrations of sodium did not show significant differences in LSI and LCI indices (Figs 2 and 3), but that potassium concentrations were significantly different (Figs 5 and 6). Therefore, we can infer that larvae mineral requirements vary during various stages of larval growth and development. Sodium and potassium ions, and their resultant mineral compositions, greatly affect the impact of hatchery water on final larval development. These ions are very important environmental macromolecules necessary for larvae haemolymph activities and functions. Haemolymph sodium and potassium contents increased nearly twofold in response to changing water salinity (Wilder et al. 1998). Also, in crustacean species during the molting stage, haemolymph osmolality and Na+ are higher than those in their culture environment, such that the water was absorbed passively by osmosis (Lignot, Cochard, Soley, Lemaire & Charmantier 1999; Wilder, Do Thi Thanh, Jasmani, Jayasankar, Kaneko, Aida, Hatta, Nemoto & Wigginton 2009). Furthermore, in M. rosenbergii-specific haemolymph FAAs are involved in mediating response to environmental water salinity and sodium concentration (Huqng, Yang, Okuno & Wilder 2001).

Several factors such as broodstock condition (Nhan et al. 2009), hatchery water calcium and magnesium concentrations (Tavabe et al. 2013a) larval nutrition (Barros & Valenti 2003a,b; Lober & Zeng 2009), brackish water sources (Tavabe et al. 2013b), rearing environment (Adhikari, Chaurasia, Naqvi & Pillai 2007; Intanai, Taylor & Whiteley 2009) and larvae density (Nhan et al. 2010) affect M. rosenbergii larval quality. For this species, larval quality parameters are assumed to be directly related to maintenance of good water quality in the culture system (Armstrong, Stephenson & Knight 1976) because its wild counterparts successfully developed in clean, brackish water estuaries. This includes monovalent ion concentrations like sodium and potassium which are important for the organisms’ many biological and physiological activities. Rafiee et al. (2013) indicated that in different sodium adsorption ratio (SAR) mediums, M. rosenbergii larval quality parameters showed different larval quality responses. For this reason, our results revealed that water quality factors, such as sodium and potassium concentrations, are very important on resultant larval quality parameters and must be carefully balanced to support efficient growth and high larval quality.

Boudour-Boucheker, Boulo, Lorin-Nebel, Elguero, Grousset, Anger, Charmantier-Daures and Charmantier (2013) showed that the ontogeny of M. amazonicum larvae osmoregulatory structures is strongly correlated with the ontogeny of the physiological processes of osmoregulation and both are correlated with its ecology. Like other Machrobrachium species, M. rosenbergii larvae are not able to survive in freshwater because they do not possess the same osmoregulatory capacity as adults (Huqng, Jayasankar, Jasmani, Saiko-Sakanaka, Wigginton & Wilder 2004). M. rosenbergii larvae, upon hatch-out at zoal stage, are exposed to brackish water and would be expected to have developed a means to osmoregulate in this environment (Wilder, Huqng, Okuno, Atmomarsono & Yang 2001). At this point, the larvae are able to exert a high degree hyper-regulated potassium control and hyporegulated sodium control in its haemolymph (Stern et al. 1987; Funge-Smith et al. 1995). Pequeux (1995) has shown that various tissues are involved in salt transportation including: the body wall, gastrointestinal tract and excretory organs. While they all play a vital role in osmoregulation, the gill epithelium is considered essential in maintaining haemolymph sodium and potassium balance in crustaceans. It was found (Singh 1980) that M. rosenbergii actually shows the greatest growth in environmental conditions which are lower than its isotonic serum concentration, so that first zoal stage of this species is able to survive for a few days in freshwater.

Larval development and quality parameters of freshwater prawn were compared between the two best combined concentrations of sodium and potassium. Interactions of these ions provided interesting results. Significant differences among the treatments showed that at 5 dph LSI and LCI and also at 10 dph, larvae survival was affected only by potassium, and that its interaction with sodium was not significant during the early larval stages (s 6–8). Na/K-ATPase enzyme is very important in crustacean osmoregulatory functions, especially the euryhaline species (Wilder et al. 2001). The Na+/K+-ATPase sodium pump provides at least part of the explanation as the driving force for trans-epithelial movement of monovalent ions across the gills and other transport tissues in crustaceans. When the external salinity is lowered from the body haemolymph at the isotonic point, the enzymatic activity of the Na+/K+-ATPase is increased in transporting tissues (Lucu & Towle

2003). Masui, Furriel, Mantelatto, McNamara and Leone (2003) reported that the potassium ion has a synergistic effect on Na/K-ATPase activity in the blue crab *Callinectes danae*. Also, Huong et al. (2004) showed that Na/K-ATPase activity is elevated during the early larval stages of *M. rosenbergii*, but decreases later on in the development process. On the other hand, potassium suppresses the sodium stimulated ATPase activity in a mixed-type kind of inhibition, whereas sodium does not exert any noticeable effect on the potassium stimulated ATPase activity (Proverbio, Zanders, Marín, Rodríguez & Proverbio 1990). Santos, Belli, Augusto, Masui, Leone, McNamara and Furriel (2007) indicated that Na/K-ATPase exchanges three intracellular Na+ ions for two extracellular K+ ions, serving as an energy transducer, which converts chemical potential energy from ATP hydrolysis into electrochemical potential energy associated with Na+ and K+ concentration gradients. Towle, Palmer and Harris (1976) confirmed that in crustacean species, Na/K-ATPase activity is highest just prior to molting and during the post-molt stages. This issue is very important for larval development because molting frequency and consequently Na/K-ATPase activity at this period is high. *Macrobrachium rosenbergii* larvae possess considerable powers of ionic regulation (Wheatly 1985; Ferraris, Estepa, De Jesus & Ladjia 1986; Tavabe et al. 2013a). With increasing water salinity, sodium and potassium concentrations, haemolymph in *M. rosenbergii* (Wilder et al. 1998) and *M. carcinus* (Moreira, Ngn, Moreira & Shumway 1988) increases slightly depending on environmental salinity. This is a critical issue affecting numerous different physiological activities and their quality in larvae. The current study results revealed that at late larval stages, significant differences among the sodium and potassium combined treatments, LSI and larvae survival parameters were affected both independently and as related to their combined interaction (Tables 6 and 7) while LCI index was not affected by the factors and their interaction (Table 8). Therefore, we can say that concentrations of sodium and potassium in hatchery water mainly affect larval physiological process and have little effect on the morphological condition. Our results showed that balance of sodium and potassium concentrations in hatchery water is very important and the Na4000/K150 combined treatment (Na/K ratio about 27) shows the best performance on *M. rosenbergii* larval quality parameters (Tables 6–8). Raizada, Javed, Ayyapan, Mukhergee, Maheshwari and Fielder (2013) concluded that inland saline water amended with potassium at a concentration of approximately 80% seawater is commercially viable for *M. rosenbergii* larviculture. Also, Damrongphol et al. (2001) indicated that the ionic requirement of newly hatched larvae of *M. rosenbergii* differed from that of developing embryos and the variation of NaCl and KCl levels significantly influenced the survival of the newly hatched larvae. Our results are in line to these reports and we emphasize that interaction of potassium versus sodium and their balance is very important in inland hatchery brackish water.

**Conclusion**

This study highlights that not only hatchery brackish water salinity is important for *M. rosenbergii* larval development but the balance of sodium and potassium ions is actually even more important. As a result of the present investigation, at early stages of larval development, the LSI, LCI and larvae survival parameters were affected only by potassium while its interaction with sodium were not significant. At late stages of the larval period, interactions between both sodium and potassium factors in brackish water only affected (P < 0.05) LSI while their interactions on LCI and survival were not significant. Also, the factors of interaction on the larval quality showed that 4000 mg L⁻¹ sodium and 150 mg L⁻¹ potassium concentrations at hatchery brackish water had the best performance for *M. rosenbergii* larviculture. This same balance at 30 dph showed the highest larvae survival (40.6 ± 2.5%) which was minimum 10% higher than for the other treatments.

**Acknowledgments**

This project was funded by research deputy administration of Natural Resources Faculty of Tehran University. Thanks are due to Fisheries Department of Natural Resources faculty of Tehran University and Department of Biology of North Carolina State University for providing experimental facilities and Ghareshirin prawn hatchery centre and Mr Doug and Johnny Barbee of D&J Prawn Farm for providing the project broodstock. Also, the authors wish to sincerely thank...
Mr Nazarzadeh, Mr Shoeiry, Dr Ghada Rabah, Mahboobeh Nejati and Jennifer Warrillow for their technical assistance with the project.

References


Mallasa M. & Valenti W.C. (1998) Comparison of artificial and natural, new and reused, brackish water for...


Uno Y. & Kwon C.S. (1969) Larval development of *Macrobrachium rosenbergii* (de Man) reared in the...


