Effects of different calcium and magnesium concentrations separately and in combination on *Macrobrachium rosenbergii* (de Man) larviculture

Kamran Rezaei Tavabe a,⁎, Gholamreza Rafiee a, Michael Frinskob, Harry Daniels c

a Fisheries Department, Natural Resources Faculty, University of Tehran, Karaj, Iran
b North Carolina Cooperative Extension Service, North Carolina State University, Trenton, NC, USA
c Department of Biology, North Carolina State University, Raleigh, NC, USA

**Abstract**

The optimum concentrations of calcium and magnesium for the combinations tested in hatchery water for *Macrobrachium rosenbergii* larviculture were evaluated. Experiments were conducted in two stages: in the first, triplicate treatments of calcium (120, 180, 240 and 300 ppm) and magnesium (300, 400, 500 and 600 ppm) were separately evaluated in a closed recirculating system (60-l). During the second stage, combinations of the two best calcium and magnesium concentrations determined in the first stage were compared. This stage used a static system (10-l) with minimal water exchange. In both stages, initial larval density was fixed at 100 larvae l⁻¹ and larval stage index, larvae dry weight, survival and time of the first postlarvae appearance were the determined parameters. The results of the first stage revealed that increasing concentrations of Ca and Mg showed a non-linear and a linear response respectively. According to these results, concentrations of 180 and 240 ppm calcium and concentrations at 300 and 400 ppm of magnesium were evaluated during the second stage of the investigation. In this stage, the results showed that after the 6th stage of larval development, interactions between both calcium and magnesium affected (P < 0.05) the larval quality parameters. The combination of these factors showed that a balance of 240 ppm calcium and 300 ppm magnesium with Mg/Ca ratio about 1.25 are optimal for larviculture. This balance at 30 days post hatch showed the highest larvae survival (40 ± 2.6%) at least 15.4% higher than the other treatments.

© 2013 Elsevier B.V. All rights reserved.

**1. Introduction**

The giant freshwater prawn (*Macrobrachium rosenbergii*) is one of the most important aquaculture species in tropical and subtropical regions of the world. The natural origin of this species is principally in southeast Asia, though during past few decades it has been introduced to numerous locations in North and South America, Africa, Europe and Asia (New, 2000). *M. rosenbergii* was first introduced to Iran from Bangladesh in 1991 (New and Kutty, 2010). Annual production of the species in Iran is estimated at about 300 tons (Iran Fisheries Organization, 2011). Though the freshwater prawn industry has grown appreciably since its inception, hatchery success has met with numerous challenges. At this time, like Vietnam (Phuong et al., 2006) a lack of stable seed and postlarvae (PL) production are main limitations for further development of the Iranian freshwater prawn industry. Larval development of *M. rosenbergii* occurs in brackish water (Cheng et al., 2003; Sandifer et al., 1975; Singh, 1980; Wickins and Beard, 1974). Breeders migrate from rivers to an estuary for spawning. After hatching, the larvae remain in this brackish water environment through metamorphosis to the PL stage. While hatcheries require brackish water for larval production, they do not necessarily have to be located near estuaries to obtain it. Suitable brackish water for larval rearing can also be obtained by mixing locally available freshwater with brine, commercial “sea salt” or mineral supplements mixed with locally available salt sources (New, 2003). Moving seawater to inland sites is expensive, and results in increased hatchery and PL production costs. In the USA, about 46% of *M. rosenbergii* hatchery expenses are related to hatchery water quality and PL production (Hanson and Sempier, 2007). To meet an ever increasing industry demand for juvenile prawn, commercial hatcheries have needed to be more efficient in their production practices, including efforts to decrease the cost and production of high quality PL under...
controlled conditions. This species easily breeds in captivity, does not require large volumes of water and its reproductive cycle may be frequently repeated over an extended period (Cavalli et al., 1999). For these reasons, inland hatcheries using artificial brackish water in recirculating systems are necessary. Main problem about prawn inland hatcheries extension is preparation of brackish water with appropriate macroelement content. Unfortunately, there is sparse information on this topic and only a few conclusive studies examining the effects of water quality on larval rearing of *M. rosenbergii* have been conducted (Valenti et al., 2010). Calcium and magnesium are very important macroelements impacting larval shell hardening, osmoregulation and growth. Concentrations of these elements vary widely in the numerous aquatic ecosystems common to the larviculture of *M. rosenbergii*. At natural habitat of the freshwater prawn larval period, near to estuaries, magnesium concentration varies from 300 to 760 ppm and calcium concentration varies from 100 to 350 ppm (Dyer, 1998; Qasim, 2003). New (2003) believes that in artificial brackish water for *M. rosenbergii* larval period, magnesium (around 500 ppm) is required in higher quantities than calcium (around 200 ppm); but he has emphasized that optimum specific ionic composition for this species larviculture has not been known.

Water quality is very important for aquatic animal growth and development; not just basic water salinity but also the specific salt composition. This includes macroelement concentrations, which are critical for the organism’s many biological and physiological activities. Studies have shown that 10–12 ppt brackish water provides the best results for freshwater prawn larvae growth and development (Rafiee et al., in press; Sandifer et al., 1975; Singh, 1980; Smith et al., 1995; Yin and Bart, 2008). The ionic composition of the water is important for all crustaceans (Greenaway, 1993). This holds true for the magnesium concentration as well as for the calcium concentration. Although maximum growth of an aquatic organism would be assumed to occur in isosmotic media, research has demonstrated this principle is not true for the giant freshwater prawn. It was found (Singh, 1980) that *M. rosenbergii* actually had maximum growth that occurs in environmental conditions lower than its isotonic serum concentration, so that first zoeal stage of this species is able to survive for a few days in freshwater. This suggests that these larvae exert a strong ability to osmoregulate (Brown et al., 2010). Calcium and magnesium are two of the most important factors of water quality for crustacean osmoregulation development (Greenaway, 1993; Smith et al., 1995). These elements also comprise a major portion of their exoskeleton with large amounts of these materials being reabsorbed during the premolt period (Greenaway, 1993). These macroelements are also significant for *M. rosenbergii* larvae development and their levels in hatchery water greatly affect molting frequency (Wildér et al., 2009), hemolymph osmolality (Wildér et al., 1998), carapace mineralization (Brown et al., 1991) and survival (Adhikari et al., 2007; Rafiee et al., in press). Although knowledge about larval osmoregulatory capability of this species is of a great importance, there is a general lack of information on this topic (Brown et al., 2010).

The present investigation was carried out to determine the effects of various concentrations of calcium and magnesium separately and in combination, on the quality and development of *M. rosenbergii* larvae. The main objectives were to determine their optimal concentrations and Mg/Ca ratio at larviculture brackish water such that this information could be used to improve hatchery success in the inland prawn hatcheries without a natural brackish water supply. The resulting knowledge may assist the development of more efficient hatchery production of *M. rosenbergii*, enhancing future development of the freshwater prawn industry.

2. 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 from April 2011 to August 2012. In the first stage, a comparison was made on the effects of various concentrations of Ca and Mg separately on freshwater prawn larval quality and development. For the second stage, these macroelements were then studied in combination. In this experiment, interactions between the two best concentrations of the macroelements from the first stage were investigated to determine their effect on larval production.

2.1. Broodstock sources

The broodstock for the initial study (n = 76) were obtained from the Ghassreshirin freshwater prawn hatchery center in Kermanshah province in western Iran. Experimental work was conducted at the aquaculture laboratory of the Fisheries Department of Tehran University. Broodstock for the second stage (n = 54) were obtained from a private farm in Kenly, North Carolina State then moved to the Lake Wheeler hatchery at North Carolina State University for testing. In the broodstock tanks, water quality parameters, photoperiod and feeding activity were monitored in accordance with recommendations for prawn broodstock (New, 2003; Nhan et al., 2010). NH4-N, NO2-N, and NO3-N levels were maintained below 0.2, 0.1, and 10.0 mg l−1 respectively. The photoperiod was set at 12 h light at an intensity of 600 lx at the water surface and temperature was maintained at 28 ± 1 °C. Prawns were fed ad libitum with a commercial formulated extruded feed (9.0 mm) for cod and haddock (Skretting Co., Canada) twice a day (at 7.00 am and 7.00 pm).

2.2. Larval collection and rearing system

In the first stage of the study, larvae collection and counting was carried out under the method described by Menasveta and Piyatiratitivokul (1980) where egg-bearing females with grayish egg masses were selected from the broodstock population and moved into larval hatching tanks. 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 in a simple water use system (New, 2003). 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 macroelement concentration, the rearing systems were managed with minimum water exchange. Water quality parameters were adjusted following Nhan et al. (2009). Total Ammonia-Nitrogen (TAN: NH4-N) and Total Nitrite-Nitrogen (NO2-N) were maintained below 0.2 and 0.1 mg l−1 respectively. Gentle aeration was applied in all rearing containers and glasses. Average water temperature and dissolved oxygen were 30 ± 1 °C and 7 ± 1 mg l−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 a day at 7.00 am and 17.00 pm at a different density for each stage of larval period (12 nauplii/ml from day 2 to day 10, 8 nauplii/ml from day 11 to 20 and 5 nauplii/ml from day 21 to metamorphosis) based on H.P. Barros and W.C. Valenti (2003). To maintain water quality, dead larvae and dead uneaten artemia nauplii
were siphoned weekly and exchanged water were adjusted same concentration of studied macroelements for each treatment. Also appropriate water quality levels were monitored by daily measuring ammonia and nitrite, paying careful attention to overfeeding. In contrast, underfeeding was also monitored to avoid reduction of growth rate and incidence of cannibalism (Nhan et al., 2010; Valentí and Daniels, 2000). To prevent underfeeding, not only artemia nauplii optimum density for each larval stage was performed but also ingestion rate of nauplii by larvae was evaluated daily.

2.3. Experimental setup and design

For the first stage (Calcium and Magnesium separately effects), larviculture systems were set up using a close recirculation water system (60-l) with bio-filter. For the second stage (Combined Ca-Mg effects) a static water system (10-l) with partial exchange of water by siphoning was used. After siphoning, the macroelement concentrations in each container were adjusted to their original levels. In both stages, initial larval density was fixed at 100 larvae l⁻¹ for each treatment. The experiments treatments were set up in triplicate at 12 ppt salinity based on (New, 2003) artificial brackish water composition, which mimic the salinity of brackish water sources freshwater prawn larvae thrive in. To adjust desired concentrations of calcium and magnesium for the treatments, their soluble salts including CaCl₂·H₂O and MgSO₄·7H₂O + MgCl₂·6H₂O (with equal weight) were used. For the first stage of study, triplicate treatments of calcium (120, 180, 240 and 300 ppm) and magnesium (300, 400, 500 and 600 ppm) were conducted and their influences were evaluated on larviculture indices separately. Afterwards, combinations of the two best concentrations of these elements determined in the first stage were performed in triplicate for each combined treatment. In all experiments initial Ca and Mg concentrations in both laboratory waters were considered then calculated to prepare these elements concentrations in the treatments.

2.4. Evaluation parameters

2.4.1. Broodstock general and reproductive parameters

Initial mean weight, total length, molting and duration of intermolt period, interspawn period, egg wet weight, fecundity, Gonadal Somatic Index (GSI) and Egg-clutch Somatic Index (ESI) of the females were recorded. Egg clusters were removed 7 days after spawning from berried females (n = 5) in order to estimate fecundity and egg wet weight. GSI and ESI indices were calculated as two important reproductive parameters for the females.

\[
\text{Fecundity} = \frac{\text{Total eggs}}{\text{female weight}} \times 100
\]

\[
\text{GSI} = \frac{\text{Gonad weight}}{\text{body weight}} \times 100
\]

Both brood females had similar general characteristics including age (7 ± 1 months), individual weight (349.5 ± 5.7 g), total length (15.6 ± 5.3 cm) and reproductive properties including intermolt period (33 ± 4 days), interspawn period (46 ± 5 days), egg wet weight (90.1 ± 6.2 g), eggs per spawning (41601 ± 6909909 female⁻¹), fecundity (1119 ± 463 egg g⁻¹ female), GSI (6.8 ± 2.3 %) and ESI (9.5 ± 2.8%).

2.4.2. Larval quality parameters

Various indices are expressed as larval quality parameters. At the present study larval dry weight, larval stage index (LSI), survival and time of the first PL appearance stage indices were assessed. 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 during the larval period and time of the first PL appearance stage was recorded in the treatments. LSI was determined according to morphological development at 5, 10, 15 and 20 dph based on the description by Uno and Kwon (1969).

\[
\text{LSI} = \frac{\text{Sn}}{N}
\]

Where

\( S_n \) is the stage of the larvae (i = 1 to 11).

\( N \) is number of larvae examined.

2.5. Statistical analysis

The data were normalized by Shapiro–Wilk test prior to further statistical analyses. In the first stage, larval dry weight, survival, LSI and time of the first PL appearance were analyzed 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, USA). In the second, two-way ANOVA was performed to evaluate the interaction of the two best concentrations of calcium and magnesium (obtained from the first stage of the study), combined with each other, on the larviculture parameters analyzed using the same software.

3. Results

Mean values (± standard deviation) for physico-chemical parameters such as temperature, pH, dissolved oxygen, ammonia-N and nitrite-N were 30 ± 1 °C, 7.6, 7 ± 1 mg l⁻¹, <0.2 mg l⁻¹ and <0.1 mg l⁻¹ respectively. Both sources of the broodstock for the study had been produced from PL of the same year and there were no significant differences between the broodstock sources in general and reproductive properties.

3.1. Calcium effect

Larvae survival at 10 dph was significantly (P < 0.05) higher at 240 and 300 ppm calcium concentrations while the same parameter at 20 and 30 dph was significantly (P < 0.05) higher at 180 and 240 ppm (Table 1). In Fig. 1, larvae dry weight is presented at three stages of the larval period in the treatments. At all stages, there were significant differences (P < 0.05) between 240 and 120 ppm treatments. LSI at 5 dph did not show significant differences among the treatments, but at 10 dph showed significant differences and faster larval development at the 240 and 300 ppm treatments (Fig. 2). Time of the first PL appearance showed only significant difference (P < 0.05) between 120 and 240 ppm treatments (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Larval age (dph)</th>
<th>Calcium concentration (ppm)</th>
<th>120</th>
<th>180</th>
<th>240</th>
<th>300</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>70 ± 5&lt;sup&gt;a&lt;/sup&gt;, 73 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86 ± 5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>84 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>48 ± 3&lt;sup&gt;a&lt;/sup&gt;, 60 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>62 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48 ± 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>25 ± 2&lt;sup&gt;a&lt;/sup&gt;, 31 ± 6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>44 ± 7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29 ± 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Time of the first PL appearance (day) 31 ± 2.7<sup>a</sup>, 27 ± 3.1<sup>a</sup> 25 ± 2.1<sup>a</sup>, 30 ± 3<sup>a</sup>
3.2. Magnesium effect

The larvae dry weight did not show significant differences among the treatments for newly hatched and 8 dph stages. But at 16 dph, there were significant differences between 400 ppm treatment versus 500 and 600 ppm treatments (Fig. 3). LSI at 5 dph did not show any significant differences among the treatments. But at 20 dph, this parameter showed significantly ($P < 0.05$) the highest value for 300 and 400 ppm treatments (Fig. 4). Also, results of time of the first PL appearance showed significant differences ($P < 0.05$) for these treatments with $25 \pm 1.6$ and $25 \pm 1.9$ days respectively (Table 2). Interestingly, larvae survival at 10 dph did not show any significant differences among the treatments, but at 30 dph, larvae survival for both 300 and 400 ppm treatments were significantly ($P < 0.05$) higher than the 500 and 600 ppm treatments (Table 2).

3.3. Combined Ca–Mg effects

Interaction of the two best combined concentrations of calcium (180 and 240 ppm) and magnesium (300 and 400 ppm) for freshwater prawn larviculture was investigated. The LSI at 10 dph showed significant differences ($P < 0.05$) among the treatments while these factors did not show significant interaction. At 15 and 20 dph this parameter did show a significant difference in the Ca 180/Mg 300 treatment providing the lowest value among the others. Also, the factors did show interaction ($P < 0.05$) for the LSI parameter at these stages (Table 3).

Calcium and magnesium factors separately and their interaction on time of the first PL appearance were significant ($P < 0.05$). The results showed that there were no significant differences for time of the first PL appearance among Ca 180/Mg 400, Ca 240/Mg 300 and Ca 240/Mg 400 treatments and significant differences ($P < 0.05$) showed only in the treatment Ca 180/Mg 300 in comparison to the others. Interaction of studied factors with each other and their effect as independently were not significant on the newly hatched larvae dry weight; but effects of calcium factor independently and its interaction with magnesium factor were significant ($P < 0.05$) for these treatments. This parameter at this stage was not significant. At 16 dph, effects of both factors and their interaction on larvae weight were significant ($P < 0.05$) but there were no significant differences among Ca 180/Mg 300, Ca 240/Mg 300 and Ca 240/Mg 400 treatments (Table 4).

Larvae survival among the treatments showed significant differences ($P < 0.05$) but calcium and magnesium interactions on larvae survival at 10 dph were not significant. This parameter at 20 and 30 dph showed significantly ($P < 0.05$) the highest value at the Ca 240/Mg 300 treatment and the factors showed interaction ($P < 0.05$) with each other on larvae survival at these stages.

4. Discussion

The results of the current study revealed that *M. rosenbergii* growth, survival, development and overall larval quality were strongly affected by different concentrations of calcium and magnesium, both separately
and in combination. The term “larval quality” generally refers to the physiological condition of the larvae and is related to survival, development and growth rates during the larval period (Racotta et al., 2003). Several factors such as broodstock condition (Cavalli et al., 1999; Nhan et al., 2009), larval nutrition (H.P. Barros and W.C. Valenti, 2003; H.P.D. Barros and W.C. Valenti, 2003; Lober and Zeng, 2009), rearing environment (Adhikari et al., 2007; Hangsapreuke et al., 2008; Rafiee et al., in press; Uno and Kwon, 1969; Yen and Batt, 2008) and larvae density (Nhan et al., 2010) affect M. rosenbergii larval quality. We have shown that water quality factors have direct and measurable effects on larval quality, survival and larval period of this species. Calcium effect shows a non-linear response of the LSI, larvae dry weight and survival to increase in calcium concentration (Figs. 1, 2 and Table 1). Both high and low calcium concentrations show negative effects on larval quality and survival. Adhikari et al. (2007) showed that in juvenile M. rosenbergii, the maximum survival was observed at a calcium hardness level of 92 ppm CaCO3, while the lowest survival was recorded at the highest calcium hardness level 384 ppm CaCO3. The results of calcium effect support these findings, but optimum Ca levels for larval period are higher than the juvenile period because the larval period takes place in brackish water where calcium concentration is higher than in freshwater. Similar results were also observed in European freshwater crayfish Austropotamobius pallipes (Greenaway, 1974), M. rosenbergii juvenile (Brown et al., 1991), red swamp crayfish Procambarus clarkii (Wheatley and Ayers, 1995) and American lobster Homarus americanus (Zhuang and Ahearn, 1996). Wilder et al. (2009) showed that calcium in the surrounding water is one of the main factors affecting molting frequency and hemolymph osmolality of M. rosenbergii. In general, calcium and magnesium are important factors that affect crustacean biological and physiological activities. In most aquatic crustacean species, these ions are readily available in the aquatic environment and are absorbed, as required, to replace those salts lost during molting (Fieber and Lutz, 1985). Furthermore, carbonates of calcium and magnesium comprise a major portion of the exoskeleton of crustaceans and a large amount of these minerals are reabsorbed during the premolt period (Greenaway, 1993). Our results showed that there were significant differences between 120 and 240 ppm calcium concentrations in time to reach PL stage and survival parameters among the treatments at different stages (dph) during larval period (Table 1). As Adhikari et al. (2007) reported, this study has also confirmed that when calcium concentration is below 92 ppm, M. rosenbergii shell hardening will be prolonged after molt. In addition, prawns exposed to low calcium concentration water are vulnerable, which may be detrimental to their health and/or efficient growth. In crustaceans, calcium homeostasis is influenced by periodic molting (Zanotto and Wheatly, 2003). As a result, the larval period is also influenced by environmental calcium concentrations and those in extracellular fluid.

In magnesium effect, as the magnesium concentration was increased, the LSI and survival showed a linear response (Table 2 and Fig. 4). Morriss and Spicer (1993) reported that high Mg2+ concentration in water and extracellular fluid in crustaceans inhibits cellular ATPase activities. Boardman and Collier (1941) also showed that a high concentration of magnesium interfered with neuromuscular transmission in the littoral crab Carcinus maenas. Conversely, Hangsapreuke et al. (2008) reported that M. rosenbergii larvae need a rather high magnesium concentration around 574 ppm in environmental water while low survival occurred during the final larval stage related to magnesium depletion in the culture water. This discrepancy in the M. rosenbergii larval requirement is likely due to mineral differences found at different culture environments and among its natural habitat. Magnesium is a powerful bioactive ion, with the activities of some enzymes dependent on specific concentration requirements. In species like M. rosenbergii, whose larvae require brackish water; high magnesium may still be necessary for a successful molt (Fieber and Lutz, 1985). Magnesium and calcium both pass through the epidermis into the hemolymph and are transported to storage or an excretion site (Greenaway and Farrelly, 1991). Our results did not show significant differences among the magnesium treatments for larvae dry weight of newly hatched or 8 dph larvae and this parameter showed only significant differences among the treatments at 16 dph larvae (Fig. 3.). This parameter also showed significant differences among calcium treatments for the newly hatched larval stage (Fig. 1). According to these results, calcium is clearly the most important macromolecule when compared to magnesium for larvae weight gain in early larval stages, while magnesium shows effect on larvae weight from stage 8 of the larval development (Fig. 3). Although stored calcium and magnesium in the cuticle are lost during molting (Adhikari et al., 2007), magnesium is the most abundant intracellular divalent cation and plays a central role in many cellular processes; this ion has been implicated in the activation of a large number of enzymes, hormonal signaling features, protein synthesis and cell division (Alvarez et al., 1987).

The combinations of calcium and magnesium provided interesting results. Significant differences in LSI at 10 dph were affected at both magnesium and calcium separately but were not affected by their combination (Table 3). On the other hand, significant differences (P < 0.05) in larvae survival at 10 d ph were only

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Survival (%) and time of the first PL appearance (mean ± SD) at different magnesium concentrations. The comparison is intergroup and different letters at each row denote significant differences (P &lt; 0.05).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larval ages (dph)</td>
<td>Magnesium concentration (ppm)</td>
</tr>
<tr>
<td>300</td>
<td>400</td>
</tr>
<tr>
<td>10</td>
<td>87 ± 11</td>
</tr>
<tr>
<td>20</td>
<td>63 ± 7b</td>
</tr>
<tr>
<td>30</td>
<td>45 ± 7b</td>
</tr>
<tr>
<td>Time of the first PL appearance (day)</td>
<td>25 ± 1.6b</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3</th>
<th>LSI (mean ± SD) at different dph of M. rosenbergii larvae reared at different combined concentrations of calcium and magnesium. The comparison is intergroup and different letters at each column denote significant differences (P &lt; 0.05).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined treatment</td>
<td>Larval age (dph)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Ca 180 × Mg 300</td>
<td>3.55 ± 0.18</td>
</tr>
<tr>
<td>Ca 180 × Mg 400</td>
<td>3.41 ± 0.27</td>
</tr>
<tr>
<td>Ca 240 × Mg 300</td>
<td>3.58 ± 0.28</td>
</tr>
<tr>
<td>Ca 240 × Mg 400</td>
<td>3.60 ± 0.15</td>
</tr>
<tr>
<td>Calcium factor</td>
<td>P = 0.428</td>
</tr>
<tr>
<td>Magnesium factor</td>
<td>P = 0.072</td>
</tr>
<tr>
<td>Interaction</td>
<td>P = 0.588</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Larvae dry weight (mean ± SD) at different dph of M. rosenbergii larvae reared at combined concentrations of calcium and magnesium. The comparison is intergroup and different letters at each column denote significant differences (P &lt; 0.05).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined treatment</td>
<td>Larval age (dph)</td>
</tr>
<tr>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Ca 180 × Mg 300</td>
<td>24.66 ± 2.08</td>
</tr>
<tr>
<td>Ca 180 × Mg 400</td>
<td>25.33 ± 3.05</td>
</tr>
<tr>
<td>Ca 240 × Mg 300</td>
<td>23.66 ± 4.04</td>
</tr>
<tr>
<td>Ca 240 × Mg 400</td>
<td>26.00 ± 2.64</td>
</tr>
<tr>
<td>Calcium factor</td>
<td>P = 0.927</td>
</tr>
<tr>
<td>Magnesium factor</td>
<td>P = 0.418</td>
</tr>
<tr>
<td>Interaction</td>
<td>P = 0.648</td>
</tr>
</tbody>
</table>
affected by calcium. This indicates that a balance of calcium and magnesium in larviculture is very important after the 6th stage (about 10 dph) of larvae development. After this stage, magnesium appears to negate the positive effects that calcium has on larval quality and survival. Greenaway (1993) showed that Ca/Mg ratios maintained by decapods are not closely linked to those in natural waters and the concentration and transport of magnesium within the body during molting are clearly under precise control. Richmond et al. (1995) reported that in crustaceans, voltage-operated Ca\(^{2+}\) channels are known to play a crucial role in regulated secretion with increasing depolarization. Silva et al. (2003) indicated that the uptake rate of calcium and magnesium ions appears to depend on the total environmental divalent ion concentration and is dictated by the electrochemical potential differences between the extracellular fluid and the water because, under certain physiological conditions, extracellular Mg\(^{2+}\) is the most abundant inhibitor of these Ca\(^{2+}\) channels; and through this action, Mg\(^{2+}\) can regulate secretion. Our results showed that significant differences in dry weight at 8 dph were apparent only for calcium while at 16 dph significant differences were observed for both calcium and magnesium concentrations and their interaction (Table 4). Larval development for *M. rosenbergii* is relatively unique; it is completed in brackish water whereas other life stages occur in freshwater. Damrongphol et al. (2001) indicated that the ionic requirement of newly hatched *M. rosenbergii* larvae differed from that of developing embryos, and variations of NaCl, KCl, or CaCl\(_2\) regardless of MgCl\(_2\) + MgSO\(_4\) is completed in brackish water whereas other life stages occur in freshwater. Mg\(^{2+}\) can regulate secretion. Our results showed that significantly high concentrations of calcium and magnesium independently showed negative effects on larval quality. Their interaction illustrated how at 10 dph of the larval period, magnesium was an inhibitor for calcium effects on larval quality. Also, their interaction on the larval quality showed that 240 ppm calcium and 300 ppm magnesium concentrations with Mg/Ca ratio about 1.25 are nearly optimum for *M. rosenbergii* culture. This balance at 30 dph showed the highest larval survival (40 ± 2.6%) which was 15.4% higher than the other treatments.

### 5. Conclusion

This study highlights the importance of appropriate concentrations of calcium and magnesium ions for *M. rosenbergii* larval development. Additionally, and more importantly, we have shown that their combination had an even greater impact on larval quality. We also demonstrated that relatively high concentrations of calcium and magnesium independently showed negative effects on larval quality. Their interaction illustrated how at 10 dph of the larval period, magnesium was an inhibitor for calcium effects on larval quality. Also, their interaction on the larval quality showed that 240 ppm calcium and 300 ppm magnesium concentrations with Mg/Ca ratio about 1.25 are nearly optimum for *M. rosenbergii* culture. This balance at 30 dph showed the highest larval survival (40 ± 2.6%) which was 15.4% higher than the other treatments.

### Acknowledgements

This project was funded by the research deputy administration of the 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 GhasreShirin prawn hatchery center 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. Shoeyi and Jennifer Warnillow for their technical assistance with the project.

### References


