Effect of Mineral Supplement (Ca, Mg and K) in Water on Developmental Stages and Survival Rate of Mud Crab (*Scylla paramamosain* Estampador, 1949) Larvae

Vutthichai Oniam[1*], Rungtiwa Konsantad[1], Jitima Suwanmala[2], Wasana Arkronrat[1] and Anurak Sookdara[1]

ABSTRACT

To improve larviculture of mud crab (*Scylla paramamosain*), we determined the impacts of calcium (Ca), magnesium (Mg) and potassium (K) supplementation in water on the development and survival rates of crab larvae in the zoea (Z1-Z5), megalopa (M) and first crab (C1) stages. Four water treatments (each with 3 replications) were used in experiments: 1) without any addition (control); 2) with 400 mg·L^{-1} Ca supplement (T1); 3) with 1,350 mg·L^{-1} Mg supplement (T2); and 4) with 380 mg·L^{-1} K supplement (T3). Minerals were added to the experimental tanks every three days. The results showed that the developmental stages of crab larvae from Z1-Z2, Z2-Z3, Z3-Z4, Z4-Z5, Z5-M and M-C1 were not significantly different in any of the treatments or the control, and the range in larval development from the Z1-C1 stages in all treatments was 24.8±0.3-25.8±0.3 days. No significant differences were observed for the survival rates of the Z1 to Z5 stages in each treatment. However, supplementation with Ca (T1) and Mg (T2) resulted in significantly higher survival rates of the M (19.8±2.8 and 16.5±2.6 %, respectively) and C1 (4.7±2.7 and 4.8±1.1 %, respectively) stages than that of the respective control (7.6±3.2 % for M stage and 1.5±0.8 % for C1 stage). On the other hand, the survival rate of the M and C1 stages in T3 (K supplement) (10.8±6.2 and 2.1±0.3 %, respectively) were not significantly different from the control. The conclusion was drawn that supplementing either Ca or Mg in rearing water increased survival rates of *S. paramamosain* larvae in the M to C1 stages.

Keywords: Larviculture, Mineral supplementation, Mud crab, *Scylla paramamosain*

INTRODUCTION

Mud crabs of the genus *Scylla* are important commercial marine crustacean species, as they represent a major fishery commodity, and are popular and widely consumed in many Asian countries including Thailand (FAO, 2013). There are four species in the genus *Scylla*, namely *S. serrata*, *S. paramamosain*, *S. olivacea* and *S. tranquebarica* (Keenan *et al*., 1998); of these, *S. paramamosain* has been targeted for aquaculture due to its abundance, fast growth and high market value (Djunaidah *et al*., 2003; Ye *et al*., 2011; Islam and Yahya, 2016). Nevertheless, aquaculture of *S. paramamosain* is very limited and commercial production still underdeveloped because the yield of this crab is low, especially based on crabs obtained under hatchery conditions.

An important obstacle of larviculture of mud crab genus *Scylla* is their low survival rates, which can be caused by several factors including cannibalism (Mirera and Moksnes, 2013; Sanda *et al*., 2021), unsuitable nutritional quality of feed

[1]Klongwan Fisheries Research Station, Faculty of Fisheries, Kasetsart University,Prachuap Khiri Khan, Thailand
[2]Department of Agricultural and Fishery Science, Faculty of Science and Technology,Prince of Songkla University, Pattani, Thailand
* Corresponding author. E-mail address: ffisvco@ku.ac.th
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(Catacutan, 2002; Nghia *et al*., 2007b), water quality (Neil *et al*., 2005; Baylon, 2013) and other factors (Hamasaki *et al*., 2002; Rabbani and Zeng, 2005; Shellet and Lovatelli, 2011; Waiho *et al*., 2018). Along with these main causes, a reduction of ions contained in the water in later stages of the nursing period also was found to result in increased mortality of young crabs (Pimsor *et al*., 2021). The depletion of the ions was caused by absorption by crabs to support their growth and survival (Nurussalam *et al*., 2017). Calcium (Ca), magnesium (Mg) and potassium (K) in the water are important factors for rearing crustaceans because they affect the molting phase (Teruaki *et al*., 2009; De Silva and Davy, 2010; Tavabe *et al*., 2013). For example, Ca is required by crustaceans in the shell formation phase. Different frequencies in the addition of Ca and Mg resulted in differences in the molting frequency in the mud crab *S. serrata*, whereby an additional 30 $mg \cdot L^{-1}$ Ca and 30 $mg \cdot L^{-1}$ Mg for every 15 days resulted in higher molting frequency than the treatments without additional Ca and Mg or the addition of 30 $mg \cdot L^{-1}$ Ca and 30 $mg \cdot L^{-1}$ Mg every 5 and 10 days (Nurussalam *et al*., 2017). Moreover, Mg in the body of the mud crab increases the absorption of Ca, and this knowledge has been applied during the shell formation phase of the edible crab *Cancer pagurus* (Fabritius *et al*., 2012). Pimsor *et al*. (2021) reported that the survival rates of blue swimming crab (*Portunus pelagicus*) larvae in the zoea IV-megalopa (79.2±24.9 %) and megalopa-first crab stages (18.0±8.1 %) cultured in Ca-supplemented water were higher than those cultured without mineral supplement (33.2±13.4 and 4.2±3.5 %, respectively). The Ca-to-Mg ratio should be 1:2.73 in the zoea IV-megalopa stages, while for the megalopa-first crab stages the ratio should be 1:2.69. Furthermore, K is important not only in maintaining water pH, but it also plays roles in energy storage and transfer, as well as in nucleotide synthesis of the crab (Romano and Zeng, 2007). However, there have been limited studies on the effect of these minerals on *S. paramamosain* larvae. Therefore, this study was conducted to investigate effects of Ca, Mg and K supplementation in the water on development and survival rate of *S. paramamosain* from zoea to first crab stages. The knowledge gained from this study may assist in increasing the survival rate of *S. paramamosain* larvae and facilitate greater larval rearing efficiency, which may eventually result in successful development of commercial crab culture.

MATERIALS AND METHODS

Study site and source of crabs used in experiments

The study was conducted at the Klongwan Fisheries Research Station, Prachuap Khiri Khan Province, Thailand. Mud crabs (*Scylla paramamosain*) with a carapace width of 6.5±0.6 cm, carapace length of 4.3±0.4 cm and body weight of 49.8±12.6 g were caught by local fishers using collapsible crab traps in the mangrove area of Pak Nam Chumphon, Chumphon Province, Thailand (10º43´-10º44´ N, 99º23´-99º24´ E) in March 2021. The crabs were reared in earthen ponds with a surface area of 1,600 m^2 at a density of 800 $crabs \cdot pond^{-1}$ or 0.5 $crab \cdot m^{-2}$. The crabs were fed with trash fish at about 5 % of body $weight \cdot day^{-1}$. To maintain good water quality, approximately half of the water in each pond was exchanged once a week. The crabs were reared until reaching sexual maturation, which took about 180 days. Berried female crabs were transferred from the earthen ponds to the hatchery and placed in 500 L fiberglass tanks to allow them to release eggs. The crabs were not fed during this period. Once hatched, all larval crabs from one female crab were collected and used for the experiment. Only strong and healthy larvae that were attracted to a light source were collected for the experiments (Djunaidah *et al*., 2003).

Experimental design and set-up and nursing protocol

The experimental crab larvae were transferred to 50 L aquarium tanks (filled with 30 ‰ filtered seawater) at a density of 100 $crabs \cdot L^{-1}$ (Nghia *et al*., 2007a). The Z stage were fed with *Artemia* nauplii at 8:00 a.m., 1:00 p.m. and 6:00 p.m., at a rate of about 5-10 $nauplii \cdot larva^{-1} \cdot day^{-1}$ until the larvae had metamorphosed to the C1 stage. In the megalopa (M) to C1 stages, a nylon net (size 12×24 inches) was provided for shelter, with two nets per aquarium tank (Pimsor *et al*., 2021).

During the larval rearing, mineral supplements were added to the water to maintain the standard mineral content of seawater every three days, with about 400 mg·L^{-1} Ca (T1), 1,350 mg·L^{-1} Mg (T2) and 380 mg·L^{-1} K (T3). These parameters were optimum for marine aquaculture, especially in crustaceans such as crabs, lobsters, and shrimps (Boyd, 2020). Its using commercial grade quantities of $CaCl_2$, $MgCl_2$ and KCl, respectively (Pimsor *et al*., 2021) with a control set without mineral supplement.

Data collection

The main data collected during the study were the development stages and survival rates in the Z1-Z5, M and C1 stages. At the time of sampling, the larvae in each stage were determined using a dissecting microscope at 10× magnification, following identification guidelines provided by Shellet and Lovatelli (2011). The larvae are considered being developed to the next stage when 50 % of a sample (n = 10) have metamorphosed. The survival rate of each Z stage was estimated based on the total number in three 100 mL aliquot water samples taken from the aquarium tank. For the M and C1 stages, the number of all crab larvae remaining were counted. The survival rate was calculated using the equation:

Survival rate (%) = number of crab larvae remaining ×100/number of initial crab larvae

During the experiment, water exchange was undertaken every three days at 20 % of the total volume during the Z1-Z5 stages, while daily water exchange (about 20 %) was carried out during the M-C1 stages. Water quality was analyzed every three days. Salinity was determined using a refractometer (Prima tech), and the pH of the water was measured using a portable pH meter (Cyber Scan pH 11). The DO and temperature of the water were measured using an oxygen probe (YSI 550A), and the total ammonia, nitrite and alkalinity of the water were determined using the indophenol blue, colorimetric, and titration methods, respectively. Water was determined for Ca and Mg using titration methods (APHA, AWWA and WEF, 2017) and for K using the method according to Sporek (1956).

Statistical analysis

Descriptive statistics of all measurements were calculated at the end of the experiments using the IBM SPSS Statistics for Windows software (version 21.0; IBM Corp., Armonk, NY. USA). All data were analyzed using one-way ANOVA, and the difference between means was tested using Duncan's multiple range test at the 95 % level of confidence.

RESULTS AND DISCUSSION

The results of water quality in this study showed the following values or ranges: salinity 30 ‰, water temperature 28.7-29.6 °C, DO 3.97-5.21 mg·L^{-1}, pH 8.28-8.55, total ammonia 0.00-0.03 mg N·L^{-1}, nitrite 0.00-0.24 mg N·L^{-1}, and alkalinity 125-147 mg·L^{-1} as $CaCO_3$. The average water conditions were not significantly different among the treatments of no mineral additions (control) and with Ca (T1), Mg (T2) and K (T3) supplements in the water ($p>0.05$). These parameters appeared not to affect the larval development or survival of this crab species (Ngnia *et al*., 2007a; Shellet and Lovatelli, 2011; Ye *et al*., 2011; Waiho *et al*., 2018).

Effect of Ca, Mg and K in water on developmental stages of crab larvae

In the current study, the developmental stages of the larvae of the mud crab *Scylla paramamosain* from the Z1-Z2, Z2-Z3, Z3-Z4, Z4-Z5, Z5-M and M-C1 stages in the control, T1, T2 and T3 were not significantly different ($p>0.05$, Table 1). In general, the development of mud crab larvae from Z1 to Z5 requires approximately 3-5 days while 7-10 days are required for the M stage prior to molting in the C1 stage (Shellet and Lovatelli, 2011; Waiho *et al*., 2018). Similar results were observed in the current study.

Effect of Ca, Mg and K in water on survival rate of crab larvae

The results revealed that the survival rate of *Scylla paramamosain* larvae from the Z1-Z5 stages in the control, T1, T2 and T3 were not

significantly different ($p>0.05$). Furthermore, the average survival rates of the M and C1 stages in the Ca-supplemented group (T1) (19.8±2.8 and 4.7±2.7 %, respectively) and the Mg-supplemented group (T2) (16.5±2.6 and 4.8±1.1 %, respectively) were not significantly different ($p>0.05$), whereas the values for both treatments were higher than for the control (7.6±3.2 and 1.5±0.8 %, respectively) ($p<0.05$), as shown in Table 2. Adding K into rearing water tended to increase survival rate as compared to the control, but the difference was not significant based on the statistical analyses ($p>0.05$).

Table 1. Period (days) of larval development (mean±SD) of mud crab *Scylla paramamosain* without mineral addition (control), with Ca supplement (T1), with Mg supplement (T2) and with K supplement (T3) in water.

Development stage	Period (days)			
	Control	T1	T2	T3
Zoea I-zoea II (Z1-Z2)	3.6±0.3	3.8±0.3	3.8±0.3	3.6±0.3
Zoea II-zoea III (Z2-Z3)	3.8±0.3	3.5±0.0	3.6±0.3	3.8±0.3
Zoea III-zoea IV (Z3-Z4)	3.3±0.3	3.8±0.3	3.5±0.0	3.6±0.3
Zoea IV-zoea V (Z4-Z5)	4.0±0.0	4.1±0.3	3.6±0.3	3.8±0.3
Zoea V-megalopa (Z5-M)	4.6±0.3	4.1±0.3	4.3±0.3	4.3±0.3
Megalopa-first crab (M-C1)	6.3±0.3	6.0±0.5	5.8±0.3	6.3±0.3
Total from Z1-C1	25.8±0.3	25.5±0.5	24.8±0.3	25.6±0.5

Note: no significant ($p>0.05$) differences among means within the same development periods

Table 2. Survival rate (mean±SD) of mud crab *Scylla paramamosain* larvae reared with no mineral addition (control), with Ca supplement (T1), with Mg supplement (T2) and with K supplement (T3) in water.

Larval stage	Survival rate (%)			
	Control	T1	T2	T3
Zoea I (Z1)	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}	100±0.0^{a}
Zoea II (Z2)	73.3±5.7^{a}	80.0±10.0^{a}	76.7±5.7^{a}	76.7±5.7^{a}
Zoea III (Z3)	63.3±15.2^{a}	66.7±5.7^{a}	70.0±10.0^{a}	70.0±10.0^{a}
Zoea IV (Z4)	53.3±15.2^{a}	53.3±5.7^{a}	60.0±10.0^{a}	60.0±10.0^{a}
Zoea V (Z5)	43.3±5.7^{a}	53.3±5.7^{a}	53.3±11.5^{a}	53.3±11.5^{a}
Megalopa (M)	7.6±3.2^{c}	19.8±2.8^{a}	16.5±2.6^{ab}	16.5±2.6^{ab}
First crab (C1)	1.5±0.8^{b}	4.7±2.7^{a}	4.8±1.1^{a}	4.8±1.1^{a}

Note: Means within a row with the same lowercase superscripts are not significantly ($p>0.05$) different.

Change of Ca, Mg and K contents in water during crab larviculture

The changes in Ca, Mg and K content in the water during the larviculture period is illustrated in Figure 1. The Ca concentration in water decreased to less than 400 mg·L^{-1} in T3 (394.3-395.5 mg·L^{-1}) and the control (395.7-397.0 mg·L^{-1}) at 9-12 days and 18-21 days of the larviculture period, respectively. Adding Mg to rearing water (T2) maintained the value of Mg (1,298.4-1,346.9 mg·L^{-1}) at nearly that of standard seawater (1,350 mg·L^{-1} Mg). Meanwhile, the value of Mg content in the control, T1 and T3 was lower than the standard value for seawater throughout the 24-day larviculture period. For K content, the concentrations in all treatments were higher than the standard value for seawater (380 mg·L^{-1} K), throughout the experiment.

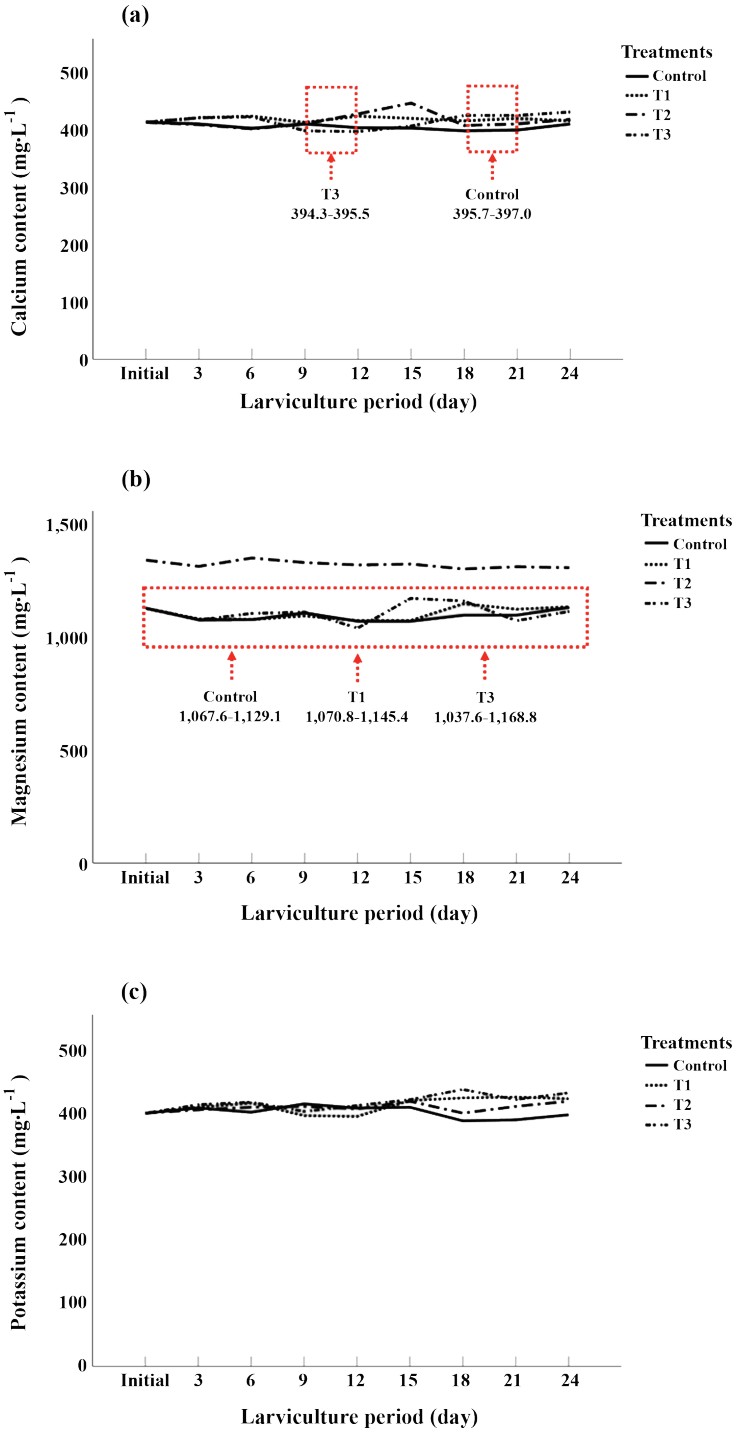

Figure 1. Change in mean calcium (a), magnesium (b) and potassium (c) content in water during larviculture of mud crab (*Scylla paramamosain*) at 30 ‰ salinity. Red boxes indicate that the level of the mineral is lower than the standard mineral content (Control = without any addition; T1 = 400 mg·L^{-1} Ca supplement; T2 = 1,350 mg·L^{-1} Mg supplement; T3 = 380 mg·L^{-1} K supplement).

The average values for Ca content in the water during the crab larviculture period were 403.0±5.3, 415.7±4.1, 413.4±13.5 and 412.8±12.3 $mg \cdot L^{-1}$; the Mg content was 1,092.3±23.6, 1,101.0± 29.5, 1,318.7±15.9 and 1,106.1±41.8 $mg \cdot L^{-1}$; and the K content was 401.4±9.1, 411.6±12.4, 408.5± 6.8 and 417.1±12.3 $mg \cdot L^{-1}$ for the control, T1, T2 and T3, respectively. There were no significant differences in the Ca content among T1, T2 and T3 ($p>0.05$), and the values for all three treatments were higher than for the control ($p<0.05$). The Mg content in T2 was significantly higher than in control, T1 and T3 ($p<0.05$). The K content in the water for T1, T2 and T3 were not significantly different ($p>0.05$), however T3 had a significantly higher K content compared to the control ($p<0.05$), as shown in Figure 2.

During the life cycle of these crabs, mortality is highest in the Z-M stages, which were reported to be major bottlenecks in *Scylla* larvae production (Hamasaki *et al*., 2002; Shellet and Lovatelli, 2011; Ye *et al*., 2011; Baylon, 2013; Waiho *et al*., 2018). Very similar results were recorded in the current study. Previous studies have reported that the causes of high mortality in brachyuran crab larvae during the transition from the Z to the M stages were bacterial and fungal infections (Morado, 2011; Wang, 2011), and molt death syndrome (Pates *et al*., 2017). However, in the present study, cannibalism was found as another main factor of larval mortality, along with molt death syndrome. Our finding was supported by Mirera and Moksnes (2013) and Sanda *et al*. (2021), who reported that although molt death syndrome may occur in the pond, cannibalism was the main factor affecting mortality during this period. Improved survival rates of *Scylla* larvae in the M to C stages may be achieved by using recirculating systems (Ngnia *et al*., 2007a).

In the current experiment, mineral supplementation in water was investigated as another option to increase the survival rates of *S. paramamosain* larvae in the M to C1 stages, especially from Ca and Mg supplementation in the water. The Ca and Mg content in the control group was significantly lower than for the Ca- and Mg-supplemented groups and for standard seawater. Therefore, it can be concluded that Ca and Mg supplementation in the water to maintain the Ca and Mg content at that of seawater (about 400 $mg \cdot L^{-1}$ Ca and 1,350 $mg \cdot L^{-1}$ Mg) every three days is an important intervention to increase the survival rate of *S. paramamosain* larvae. Owing to the apparent role of Ca in molting, Mg content in the water may have only minor effect on improved survival of the crab larvae, whereas K content has no effect on the survival rate of crab larvae in M and C1 stages. It is well known that crab larvae need Ca before molting in M and C1 stages for the formation of the carapace. Calcium in the seawater binds to CO_2 forming $CaCO_3$, which plays essential roles in all the cells in soft tissue and the harder exoskeleton for molting (Bogart *et al*., 2016). Similar results for other crustacean species have been reported, including the giant freshwater prawn *Macrobrachium rosenbergii* (Tavabe *et al*., 2013), the edible crab *Podophthalmus vigil* (Soundarapandian *et al*., 2014), the white shrimp *Litopenaeus vannamei* (Valenzuela-Madrigal *et al*., 2017), the mud crab *S. serrata* (Nurussalam *et al*., 2017), and the blue swimming crab *Portunus pelagicus* (Pimsor *et al*., 2021).

Based on the current results, the highest survival of *S. paramamosain* larvae in the M to C1 stages occurred from using a Ca-to-Mg-to-K ratio of about 1:3:1 (ranging from 1:2.6:1 to 1:3.2:1). However, each species of crustaceans may have different mineral ratio requirements; for instance, the highest survival of the giant freshwater prawn *M. rosenbergii* occurred with a Ca-to-Mg ratio of 1:1.25 (Tavabe *et al*., 2013), whereas for the white shrimp *L. vannamei*, the ratio was 1:3 (Valenzuela-Madrigal *et al*., 2017). Pimsor *et al*. (2021) reported that the Ca-to-Mg ratio for blue swimming crab *P. pelagicus* larvae should be 1:2.7 in the Z4-M stages and 1:2.6 in the M-C1 stages. Notably, in general, the Ca-to-K ratio in coastal water is 1:1, so that differences from this can be amended by the addition of K instead of Ca.

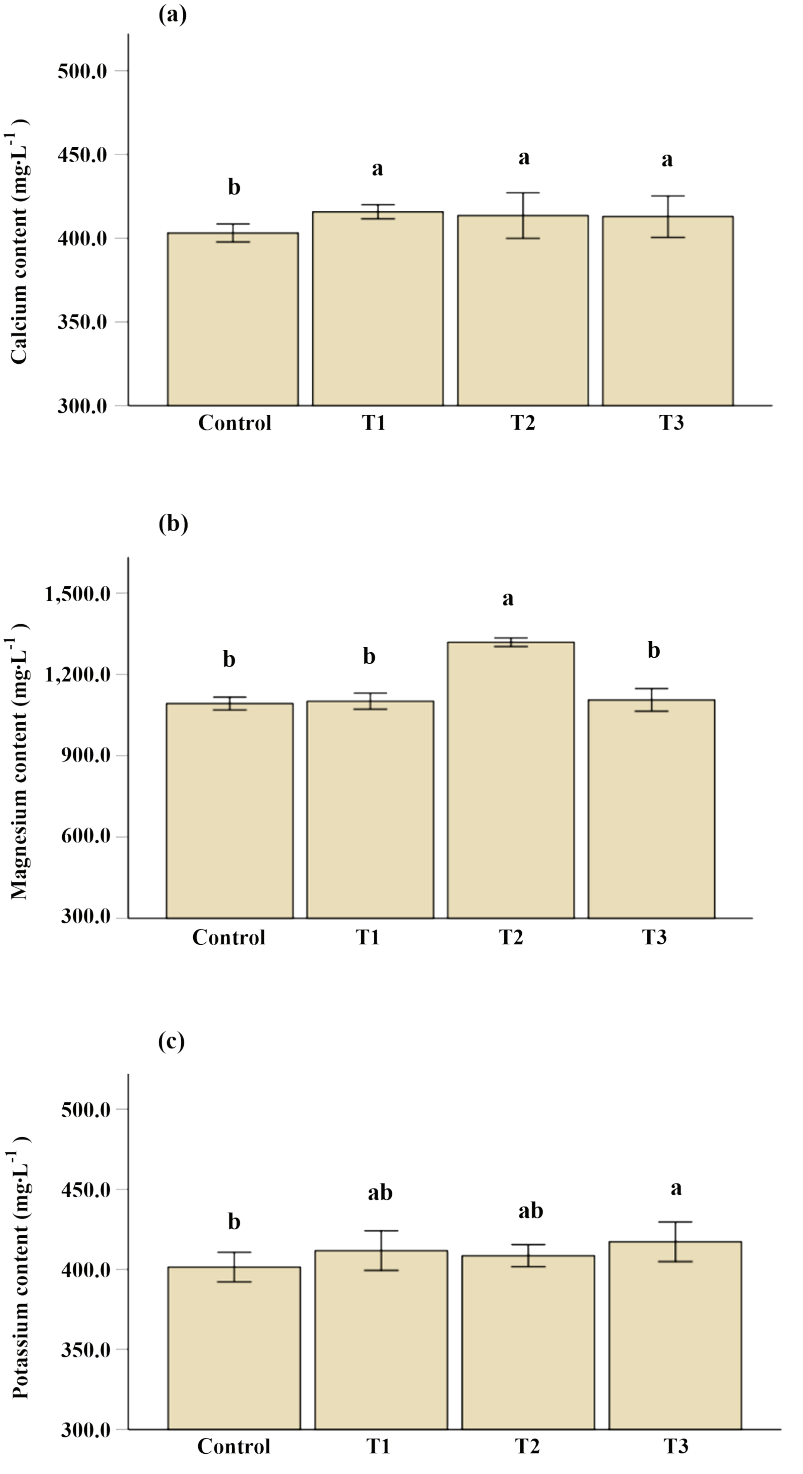

Figure 2. Mean values of calcium (a), magnesium (b) and potassium (c) content in water averaged across the nursing period of mud crab (*Scylla paramamosain*) at 30 ‰ salinity. Different lowercase letters above bars indicate significant ($p<0.05$) differences between treatments. Error bars indicate SD.

The frequency and the dosage of the addition of minerals can be adjusted to ensure excess addition of minerals does not adversely affect the mineral ratio, which in turn could harm the water conditions and growth of the aquatic animal being reared (Suguna, 2020). Similarly, Nurussalam *et al*. (2017) reported that the optimum frequency for the addition of Ca and Mg in a recirculation system to increase the production and formation of the mud crab *S. serrata* shell was every 15 days with a concentration of 30 $mg \cdot L^{-1}$; this strategy produced significantly different results from those with no addition of Ca or Mg, or from the addition of Ca and Mg every 5 and 10 days. The results of the current study indicated that analysis of the Ca, Mg and K content in the water during *S. paramamosain* larviculture is important, and that supplements of Ca and Mg could be added to the water every three days to maintain suitable Ca-Mg-K levels to increase the survival of crab larvae in the M and C1 stages. This offers another solution to improve management during crab larviculture. Notably, the survival rates of crab larvae in Z1 to C1 stages of the control treatment in the present study (1.5±0.8 %) were not different from the other studies (e.g., 0.2-2.5 %, Syafaat *et al*., 2021). However, the study of other minerals is necessary, considering the impacts of their concentration on the development and survival rate of *Scylla* larvae in the Z1-Z5 stages. There are still other factors that were not examined in the present study, especially Cl, HCO_3, CO_3 and the residual ions, HCO_3 and CO_3 in medium at termination of the experiment. This is an interesting point for future study and will be useful in the future development of strategies for improvement of crab larviculture.

CONCLUSION

Under the experimental conditions, supplements of Ca, Mg and K in the water did not affect the larval developmental stage of the mud crab *Scylla paramamosain* in the Z to C1 stages. However, supplements of Ca and Mg in the water increased the survival rate of the crab in the M and C1 stages over the crabs reared without any mineral addition to the water. Thus, Ca and Mg supplementation in the water is important to increase the survival rate of *S. paramamosain* larvae, with a recommended Ca-to-Mg-to-K ratio of 1:3:1. This could be an alternative option for increasing the larval survival rate of this crab species and facilitate greater larval rearing efficiency in the future.

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