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RESEARCH PAPER

Effects of Nursing Methods, Astaxanthin Supplementation and Water Quality on the Survival of Blue Swimming Crablets (*Portunus pelagicus,* Linnaeus, 1758)

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Abstract

The present research investigated the effects of nursing on blue swimming cablets (*Portunus pelagicus* Linnaeus, 1758) between Cup and Substrate methods for 35 days on the survival and growth with and without astaxanthin supplementation. Four treatments (Cup + Asta), (only Cup), (Substrate + Asta) and (only Substrate) were replicated three times in plastic tanks (500 L). Growth performance and the water quality parameters were monitored weekly. Results showed that there was no significant difference ($P>0.05$) between with and without astaxanthin supplementation in terms of survival, weight and carapace width. The survival of crablets decreased linearly over the nursing period and final survival of crablets in the Cup method (63.0%) remained three times higher ($P<0.05$) than the Substrate method (21.3%). The final average survival ranged from 16.7 to 68.5%. Among the water quality parameters, temperature, pH, Nitrite (NO_2) and Nitrate (NO_3) had negative linear correlations with survival, while dissolved oxygen (DO) had positive linear correlations. Average survival at 30 ppt salinity was 1.67 time higher than at 29 ppt salinity. In conclusion, to increase crablet survival during nursing, maintaining suitable environmental conditions and using Cup method are suitable for preventing cannibalism.

Introduction

Crabs are high-value seafood items marketed around the world (Hungria, dos Santos Tavares, Pereira, de Assis Teixeira da Silva & Ostrensky, 2017). Blue swimming crab, *Portunus pelagicus* (Linnaeus, 1758) is one of the most important species, which is widespread across the Indo-Pacific region, including Southeast Asia. The world aquaculture production of blue swimming crabs increased nearly 50% from 184,249 tons in 2010 to 265,898 tons in 2016 (FAO, 2018). However, due to low crablet survival and other problems, expansion of its farming is facing difficulties. Wild stock is declining along with other fishery resources. There is lack of essential biological information required for the development of appropriate grow-out techniques. Several attempts have been made to increase survival and production efficiency of portunids with a view to meeting their rapidly increasing demand. However, low survival of crablets during early stages, which may drop down to 3% is the main obstacle for the growth of crab aquaculture (Kedmuean, Kaew & Jithman, 2004). Development of a practical technique for the nursing of crablets that could improve survival would be an important breakthrough. Low survival in crablet phase is mainly due to cannibalism. Degree of cannibalism depends on body size variation, moult stage and refuge availability. (Marshall, Warburton, Paterson & Mann,

2005; Luppi, Spivak & Anger, 2001). Other factors favoring cannibalism are high stocking density and shortages of suitable food (natural live food or artificial powders) (Moller, Lee, Paterson & Mann, 2008; Roslan, Taher, Ehteshamei, Arshad & Romano, 2016). There is a lack of information about the type of food (color, shape, smell etc.) that crablets prefer. Like any other animals, young crablets are also weak and vulnerable due to lack of developed body functions and immune systems. Supplementation of some nutrients or feed additives might have positive impacts on the intake of external food and immune system, which might help reduce cannibalism. Some researchers thought astaxanthin would be one of them (Han *et al*., 2018) as it is a member of carotenoids and has positive impacts on some aquatic animals. For example, supplementation of astaxanthin in red king crab (*Paralithodes camtschaticus*) had stimulated immune system resulting in the increased survival rate (Daly, Swingle & Eckert, 2013). In Pacific whiteleg shrimp (*Litopenaeus vannamei*) astaxanthin improved survival and enhanced resistance to several stress conditions, such as low dissolved oxygen, low salinity, low temperature, and ammonia stress (Chuchird, Rorkwiree & Rairat, 2015). Therefore, the present experiment was conducted to investigate the effects of nursing systems (with Cup and Substrates), the supplementation of astaxanthin and water quality on the survival and growth of crablets.

Materials and Methods

Source of Crablets

A total of 216 Blue swimming crablets were acquired from Klongwan Fisheries Research Station, Prachuap Khiri Khan Province, Thailand (11°45'15.9"N 99°47'30.4"E) in February 2017. The weight of crablets ranged from 0.1–0.25 g with size about 1 cm (carapace width) and 10 days metamorphosed from megalopa. The crablets were transported at a density of 10 crablets/ litre of seawater with sea pine leaves (act as shelters) using a vehicle to the Silpakorn Aquaculture Research Station (Thailand) and stocked into 12 experimental plastic tanks (109 cm × 61.5cm × 53 cm) with salinity 30 ppt in 500 L capacity (Figure 1).

Experimental Design and Culture Method

The crablets were acclimatized for a week before stocking into 12 experimental plastic tanks with 18 crablets per tank. The trial was carried out using four treatments with three replicates. The four treatments were as follows:

1. Cup method with 6 g/kg of astaxanthin (Cup + Asta)
2. Cup method without astaxanthin supplementation (Only Cup)

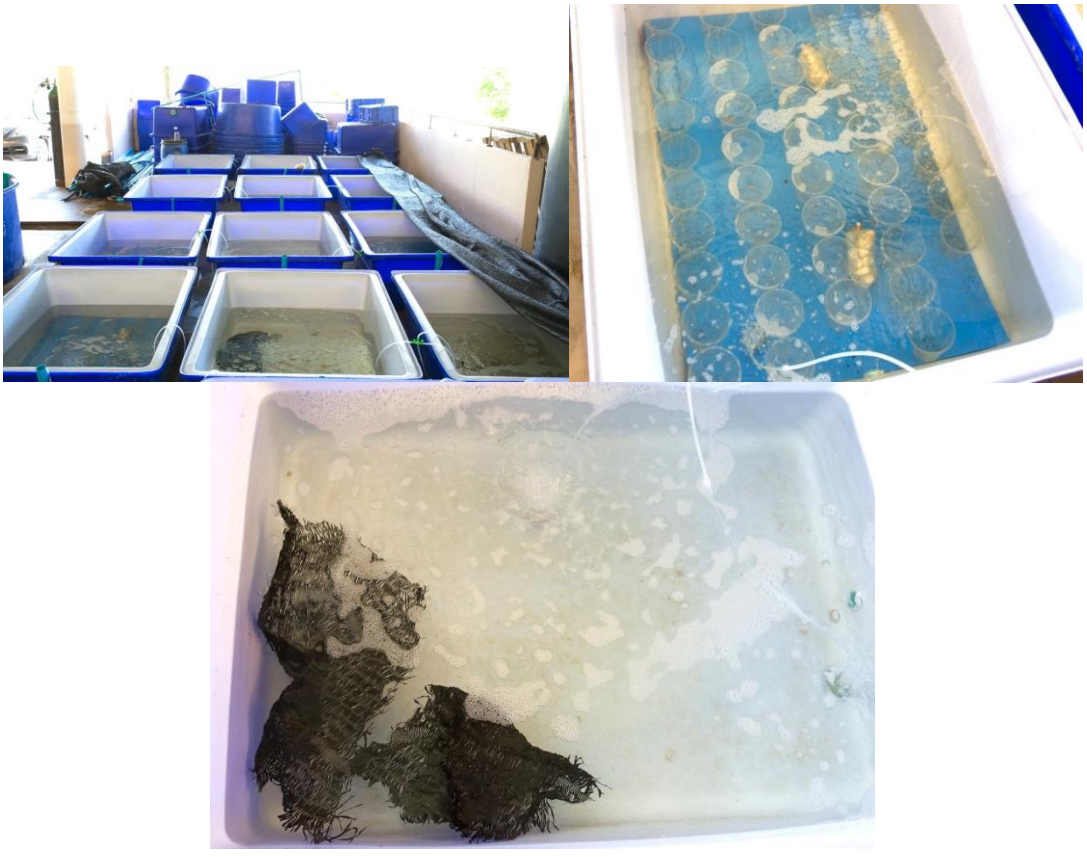

Figure 1. Experimental system (top left) of crablet nursing: with Cup (top right) and Substrate (bottom).

3. Substrate method with 6 g/kg of astaxanthin (Only Asta)
4. Substrate method without astaxanthin supplementation (Control)

All treatment tanks were kept in a temperature controlled room to maintain the water temperature range of 26-30 ^{o}C. They had a continuous recirculation system that generated a daily turnover volume of 400% water via a flow-through filtration tank (50 L). Aeration was provided in all replicates for the whole duration of the experiment and fed 2 times a day with post-larva shrimp pellet feed. The trial ran for 35 days. Every week all live crablets were weighed, measured by photography using imageJ program (software name) and survival was recorded too. Crablets were nursed using Substrate method. In this trial, a black plastic shading net were cut into 5 pieces, 90 cm^2 each to provide shelter as complex structures with interstitial spaces which reduces contacts with each other (Daly, Swingle, & Eckert, 2009). As a new method, a plastic cup (0.5 L) was used for each individual crablet with bottom diameter of 5.5 cm height 14.5 cm. Each cup was covered with its lids after placing a crablet in. Several holes were made around the cups for water ventilation then placed on the plastic board within the tank filled with 150 L of seawater. Pellet feed used for the experiment contained 43% crude protein, 4% crude lipid, 4% crude fiber and 12% moisture (Kung best company). Feeding regime was once a day at 3 pm with 30% of wet body weight. Uneaten food and wastes were siphoned from rearing tanks daily. Organic *Haematococcus pluvialis* extract powder containing 10% astaxanthin (ASTA) certified analysis by Herbal Cure Company was used at the recommended dose of 600 mg per 100 g pellet feed (i.e. 6 g/kg feed) and diluted with water then sprayed on to the feed and coated with 5% chitosan solution for those treatments as per recommendation. Water quality indicators such as dissolved oxygen alkalinity, salinity, pH, ammonia, Nitrite and Nitrate were monitored weekly and 80% water was changed every 3 days intervals.

Statistical Analysis

As survival, weight and carapace width were associated with each other, multivariate analysis of variance (MANOVA) was performed to determine the main effects of nursing method and astaxanthin supplementation. Their interactions were also analyzed using water quality parameters as covariates. Trends of survival and growth over the experimental period were analyzed using regression. A Correlation test was done to determine the associations among survival, weight gain, carapace gain and water quality parameters. These statistical tools were applied using SPSS ver. 18 for Windows considering the differences are significant at $P<0.05$.

Results

Survival and Growth performances

Final survival, carapace width and weight of the crablets are presented in Table 1.

Survival rate

Results showed that the mean survival of crablets ranged from 16.7 ± 8.5 to 68.5 ± 8.1 (Table 1 & Figure 2) showing significant responses to the treatments. However, the significant factor ($P<0.05$) influencing the survival was the method of nursing i.e. with the use of Cup vs. Substrate method. Cup method improved the final survival of crablets by about three folds i.e. 63.0% vs 21.3% as compared to the Substrate method (Table 1 & Figure 2). Results showed that the survival of crablets did not differ significantly ($P>0.05$) due to supplementation of astaxanthin in both the nursing methods. There was also no interaction effect between nursing methods and the astaxanthin ($P>0.05$).

The survival rates between Cup and Substrate method started showing wide differences from the third week of the experiment (Figure 2). Survival rates of crablets in each treatment declined linearly with the

Table 1. Crablet survival, final weights and carapace width after rearing for 35 days

Parameters	Nursing methods	Astaxanthin supplementation		Mean ± SE
		With	Without	
Survival (%)	Cup	68.5 ± 8.1	57.4 ± 3.7	63.0^{a} ± 5.6
	Substrate	25.9 ± 9.8	16.7± 8.5	21.3^{b} ± 4.6
	Mean ± SE	**47.2^{a} ± 21.3**	**37.0^{a} ± 20.4**	
Final weight (g)	Cup	1.28 ± 0.39	1.04 ± 0.15	1.16^{a} ± 0.12
	Substrate	2.79 ± 0.71	5.36 ± 1.80	4.07^{b} ± 1.29
	Mean ± SE	**2.03^{a} ± 0.75**	**3.20^{a} ± 2.16**	
Carapace width (cm)	Cup	2.42 ± 0.26	2.25 ± 0.10	2.33^{a} ± 0.08
	Substrate	3.04 ± 0.30	3.97 ± 0.58	3.50^{b} ± 0.47
	Mean ± SE	**2.73^{a} ± 0.31**	**3.11^{a} ± 0.86**	

Values in the same column with different superscript are significant different at $P<0.05$. **Significant different at $P< 0.05$, ^{ns} no significant at $P> 0.05$**

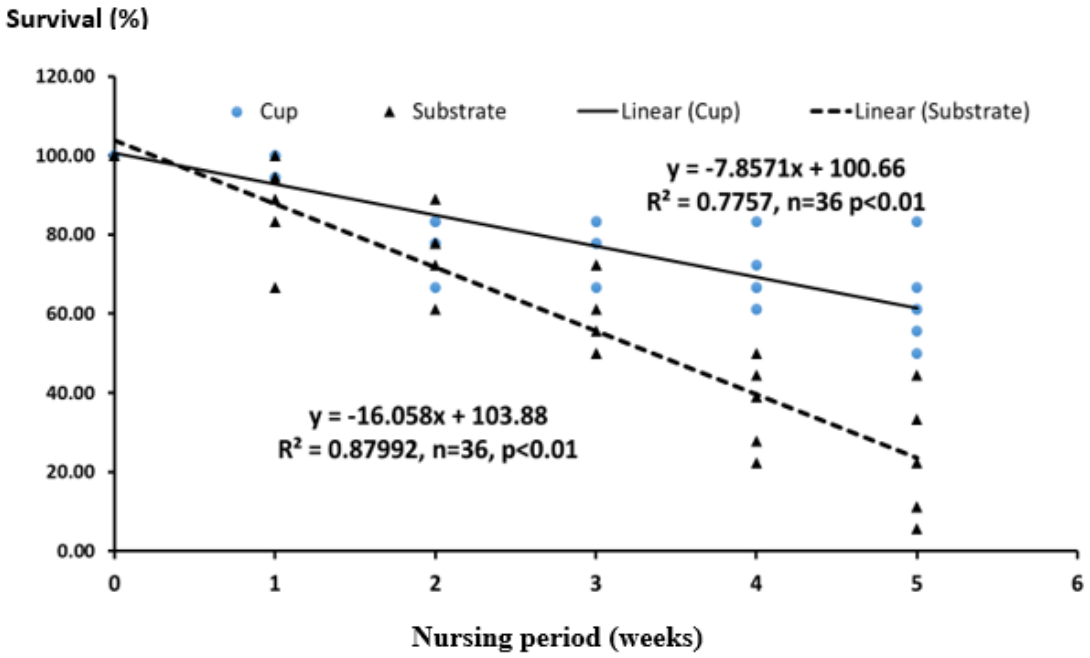

Figure 2. Survival of crablets (%) during 35 day nursing using Cup and Substrate methods.

time of nursing with R^2 values well over 90% (Figure 2). The rate of decline i.e. average slope coefficient for Substrate method was -16.06 so the survival decreased by about 16% for each week of rearing. Meanwhile, the slope coefficient for the Cup method was -7.9 that means the survival decreased by about 8% for each week of rearing, which is half of that of Substrate method.

Weight and Carapace Width

Results showed that crablets in Substrate method (with and without astaxanthin) started showing higher weight than in the Cup method (Figure 3) after the third week, which is almost three times higher weight as compared to that of crablets in the Cup method on the last week (Table 1). The result also demonstrated that crablets from Substrate method had higher carapace width than from the Cup method after the third week (Figure 4) as in the case of weight then at the final week reaching almost 1.5 times larger in terms of carapace width than from the Cup method (Table 1). Simple linear regression between crablet weight and carapace width with the period of rearing weeks showed a strong positive relationship (Figure 3 and 4) with high R^2 values at 0.01 level of significance. The crablets in the case of Cup method had linear but slow growth in weights and carapace widths whereas in case of Substrate method, growth was faster with exponential. More interestingly, carapace width of crablets from the groups supplemented with astaxanthin were significantly higher than those of crablets nursed in only Cup method at the end of 2^{nd}, 3^{rd} and 4^{th} week of samplings whereas weights were significantly higher ($P<0.05$) only at the end of 3^{rd} and 4^{th} week. Astaxanthin showed some indication of impacts on growth performance. However, with and without astaxanthin supplementation did not show any significant differences ($P>0.05$).

Water parameters analysis

Temperature and Survival

Regression analysis showed that survival declined linearly with the temperature of the water (Figure 5). Although, the temperature of water had a narrow range i.e. 25-28 ^{O}C. the trend was clear and highly significant ($P<0.01$). The declining survival rate with each unit of temperature (^{O}C) was about 15% on the Cup method whereas it was almost triple in the case of Substrate method. Highest survival occurred at lower temperatures i.e. 25-26 ^{O}C and lowest survivals were at higher temperature i.e. at 28 ^{O}C. In Cup method, survival was less varied whereas in Substrate method, it varied highly dropping down to the lowest levels.

Salinity and Survival

Although the salinity range of experiments was very close i.e. 29 and 30 ppt respectively, average survival rates at these salinities differed significantly ($P<0.01$) showing 1.67 times higher survival at 30 ppt ($88.8 \pm 2.2\%$) as compared to 29 ppt ($53.2 \pm 5.5\%$).

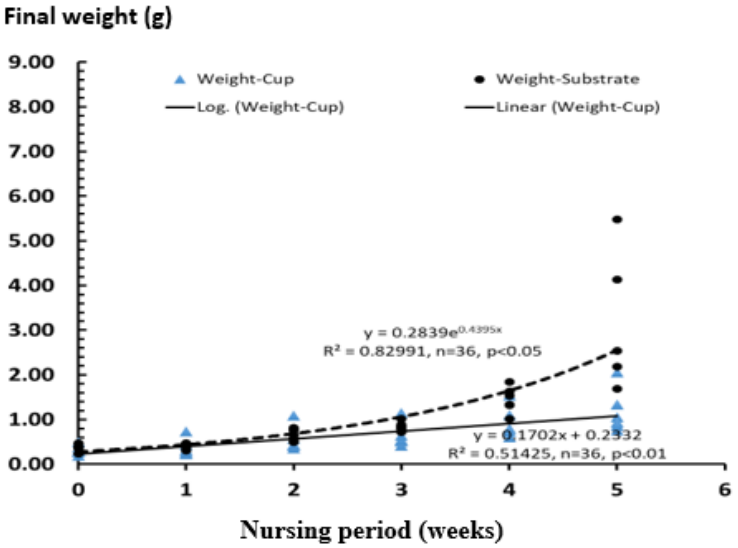

Figure 3. The average weight (g) of crablets from all four treatments.

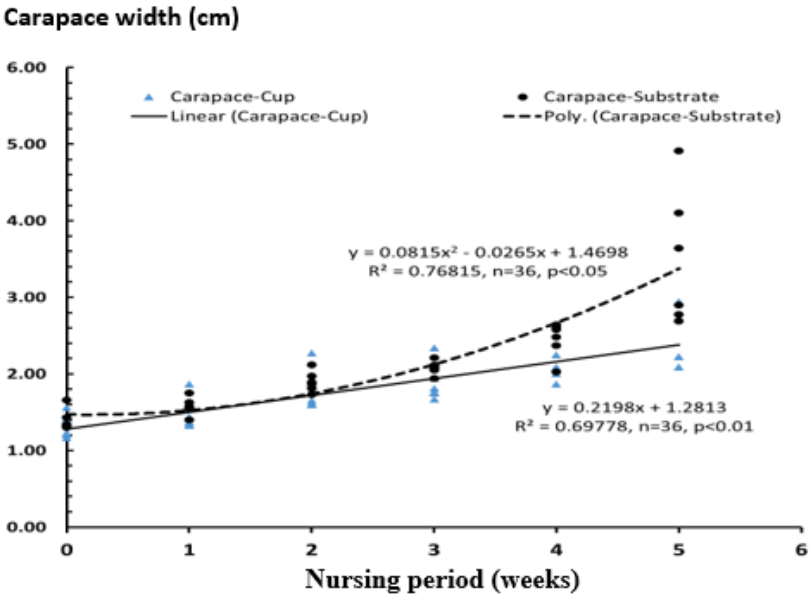

Figure 4. The average of carapace width (cm) for five week.

DO (Dissolved Oxygen) and Survival

As tanks were aerated, DO of the water had a narrow range i.e. 5.3-6.1 mg/L; however, regression analysis showed a linear increment by 28% in survival per unit of DO level in the Cup method (Figure 7), whereas the survival peaked at 5.8 mg/L in case of Substrate method.

pH and Survival

Regression analysis showed negative correlation of survival between pH levels, even though it had a small range i.e. pH 7.91-8.15. The rate of decline in the Substrate method was steeper and reaching the lowest level at the highest pH (Figure 8). The analysis showed the pH above 8.0 was likely to be detrimental

Alkalinity and Survival

The correlation between Cup and Substrate method with survival showed no significant difference ($P>0.05$) which indicated that there was no significant effect on survival rate in blue swimming crablets with alkalinity range of 130-150 mg/L

Nitrogenous wastes and Survival

Total Ammonia Nitrogen (TAN), did not have any correlation with crablet survival. But the Nitrate and Nitrite nitrogen concentrations had highly significant ($P<0.01$) and negatively correlated with the survival rate. The Nitrate nitrogen (NO_3) ranged from 0.01-3.28 mg/L and the Nitrite nitrogen (NO_2) ranged from 0-0.12 mg/L. The rate of decline in survival was about 12% and

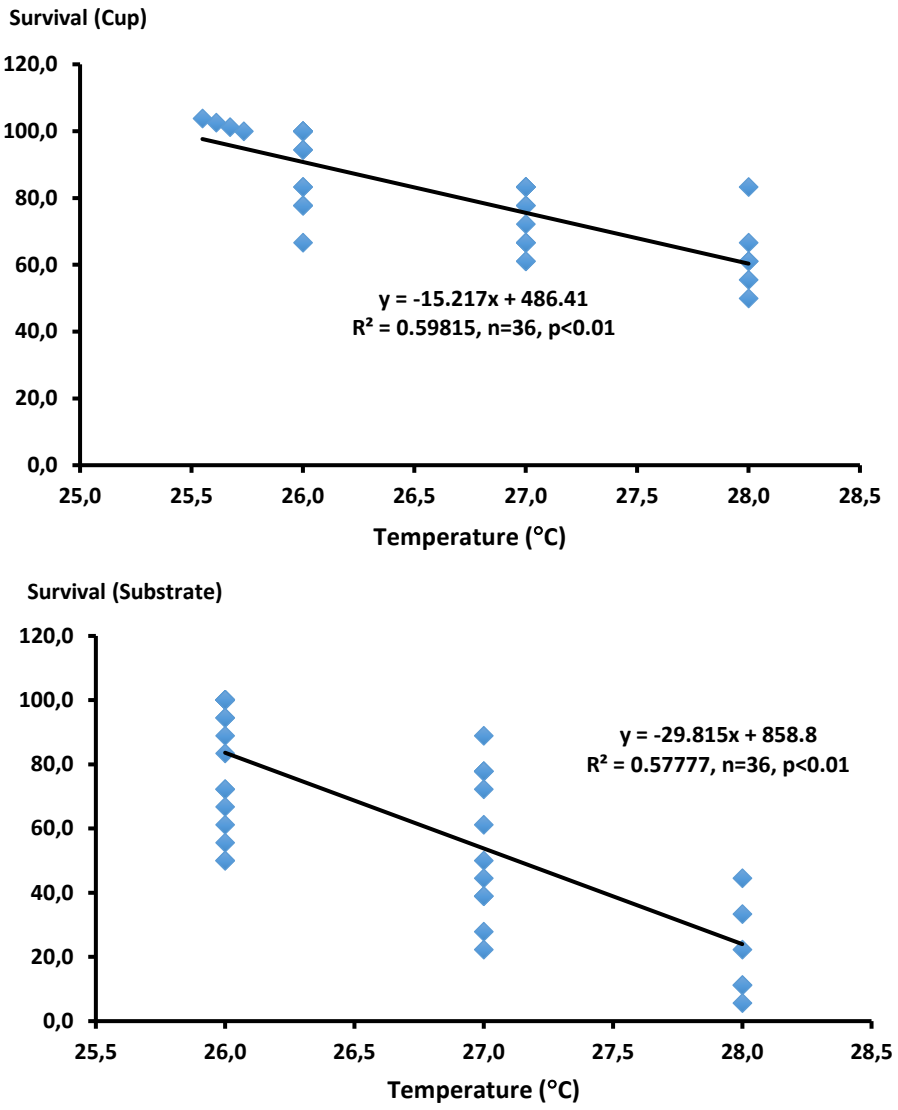

Figure 5. Survival of crablets (%) at varying water temperature (°C) during nursing with Cup (top) and Substrate (down) methods.

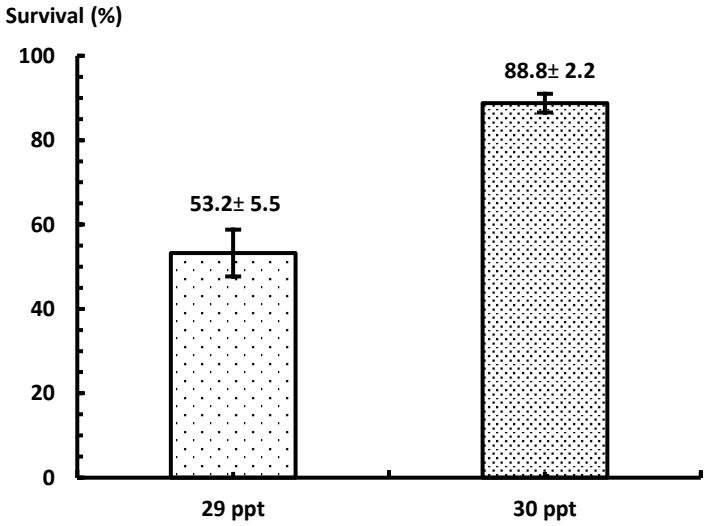

Figure 6. Survival of crablets (%) at salinities 29 and 30 ppt.

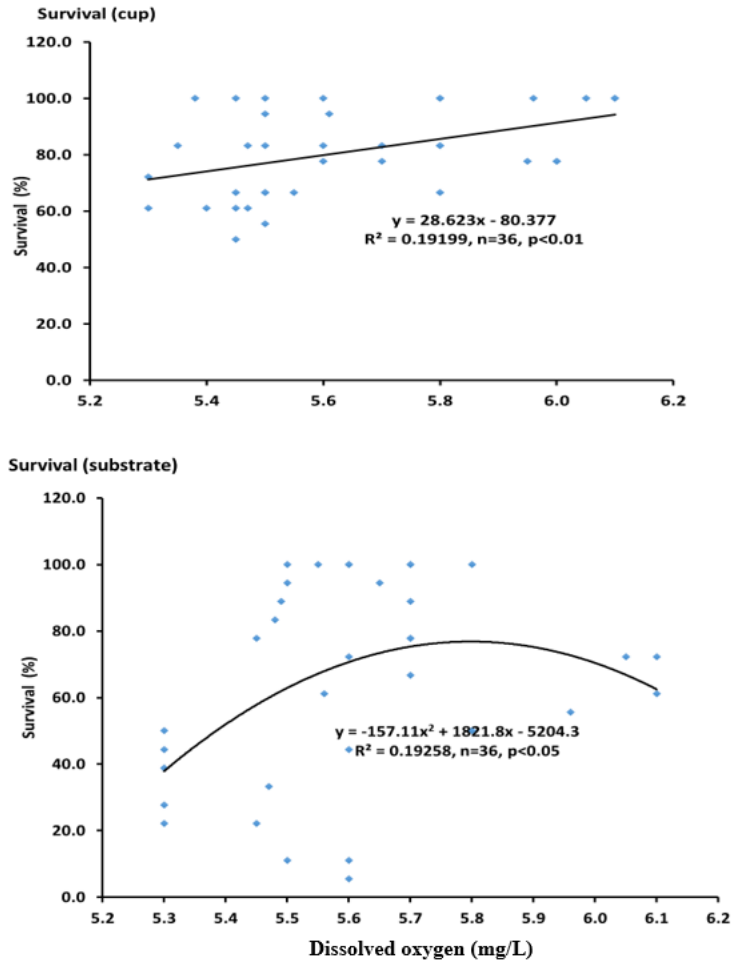

Figure 7. Correlation between survival (%) and dissolved oxygen (mg/L).

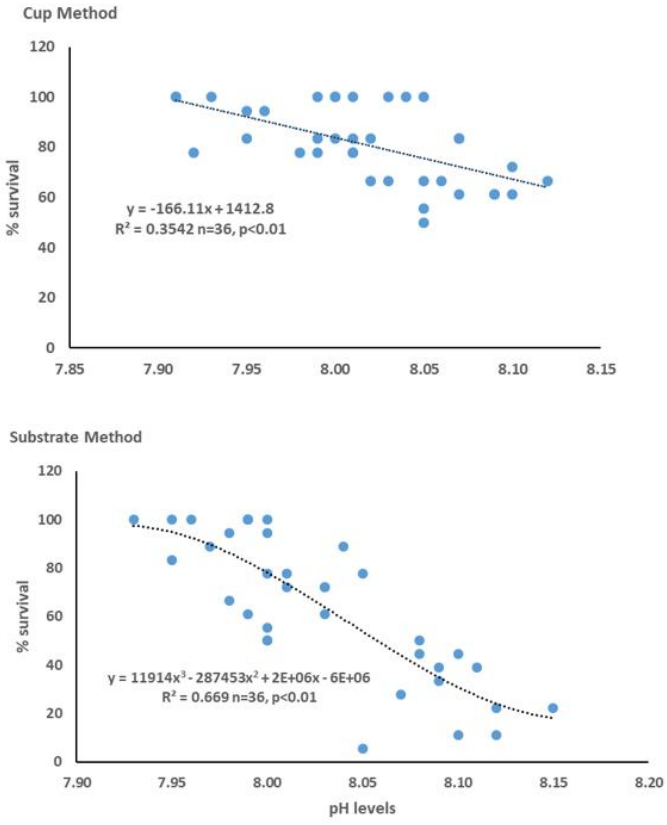

Figure 8. Survival of crablets (%) at varying pH during nursing with Cup (top) and Substrate (down) methods.

20% per unit level of $N0_3$ (1 mg/L) in case of Cup and Substrate method respectively (Figure 9-10). In the Substrate method, survival rate dropped very low when NO_3 reached 2 mg/L, whereas survival in the Cup method remained high when NO_3 reached up to 3.28 mg/L

Discussion

For the reduction of dependency on the wild stock of crabs, development of crab culture is necessary. To date, the main problem remains the poor survival of crablets during nursing at young stages (Fielder & Allan, 2004; Waiho *et al*., 2018). The poor survival is considered mainly due to cannibalism but it is not fully understood yet (Chen, *et al*., 2014). Besides shrimp farming, appropriate nursing method would also ensure high survival of crablets to help in stocking the natural water bodies for conservation purpose. Various factors influence its survival e.g. geography, climatic patterns, species culture, feeding regimes and salinities (Dan & Hamasaki, 2011; Azra & Ikhwanuddin, 2015). Some efforts have been made to identify the factors, which can be manipulated in laboratories such as tank colour, stocking density, antibiotic administration and water exchange. However, only some of them have been found to improve the survival such as dark tank color (Ikhwanuddin, Monsoor, Bolong & Long, 2012), stocking density, artificial foods and feed supplements (Heasman & Felder, 1983; Williams, Wood, Dalliston, Shelley, & Kuo, 1999; Hamasaki, Suprayudi & Takeuchi, 2002; Mann, Asakawa, Kelly, Lindsay & Paterson, 2007). The present experiment was an attempt towards developing a reliable nursing system such as the use of Cups and application of supplements e.g. astaxanthin with a view to improving crablet survival by minimizing or avoiding cannibalism. Results clearly revealed that the use of cup has remarkable increase (3 folds) in the survival of crablets during the period of 35 days of nursing. It is due mainly to the reduction in cannibalism when they are reared individually in cups without allowing them to attack. However, this improvement in survival may not be applicable as such for large scale operations as it is time consuming, laborious, costly; therefore, less practical. Normally, crabs are nursed using substrates such as tree leaves assuming that they provide hiding substrates, seagrass as in the case of the natural environment (Nitiratsuwan, Nitithamyong, Chiayvareesajja & Somboonsuke, 2010) which help reduce cannibalism. However, even then, it remains around 10% during the first stage and 20-30% during the larval stage (Hamasaki, Obata, Dan, & Kitada, 2011). Normal practice is to have more crab broods and stocking far more megalopae to compensate for the huge mortality losses. If an individual Cup method improves survival by 3-4 folds then, this method may need to be considered and further developed to make it more practical.

Use of Cup method increased survival but growth measured in terms of weight and carapace width in Cup method was hampered showing inferior compared to the Substrate method. This indicated that crablets need additional food or nutrients to support growth or need more space to go around and find more food available in the system (Heasman & Felder, 1983). As in the case of Substrate-method or non-captive system, crabs had the chance to feed on other feeds including vulnerable crabs especially during molting time (cannibalism) in a vast area which might have resulted in faster growth. In Cup method food is limited and dependent on the food offered based on the schedule. Therefore, even though the Cup method provides a promising technique for high crablet survival, it still needs more ways to improve growth to be regarded as a successful method. From this point of view, finding the appropriate feeds, optimal feeding regime and space for each individual should be topics of further research to achieve high growth along with high survival simultaneously. Type of feeds are obviously an area of interest. Various feeds and feed supplements have been tried. For example, a supplementation of astaxanthin in feed was thought to be beneficial as was found to have good impacts on kuruma shrimp juveniles, *Marsupenaeus japonicas* (Wang *et al.,* 2018). However, in this experiment the result was similar to Montakan, Nonglak and Kobsak, (2014) who reported that the effect of dietary pigment sources on growth, survival rate, molting and carotenoids profile of blue swimming crab (*Portunus pelagicus*) was not significantly different ($P<0.05$). Han, Wang, Li Wang, Yang and Zheng (2018) has also reported to have no impacts on growth performance when astaxanthin was fed up to 120 mg/kg diet. Likewise, a study indicated that there were no significant different ($P>0.05$) in both vitamin E (200, 400 and 600 ppm) and astaxanthin (100, 300 and 500 ppm) on growth and survival rate of the whiteleg shrimp (*Litopenaeus vannamei*) (Supasai, 2004). Astaxanthin supplementation was reported to improve survival of several aquatic animals but for blue swimming crab there was no tangible effects except some indications of benefits in growth in terms of weight and carapace width. Therefore, more research with higher dose or in combination with other supplements is needed to investigate further.

Present findings also showed that not only cannibalism but also several water quality parameters are very important to maintain high survival as indicated by the declining survival even when they were kept in the individual cups. It was found that survival was higher at low temperature whereas for the growth it was found to be the opposite. At high temperature, survival was low therefore, remaining crablets had higher body weight. Some authors also have reported higher temperature enhances the growth (Yuan, *et al*., 2017, Ruscoe, Shelley & Williams, 2018). However, higher temperature (>32°C) can be detrimental as reported by

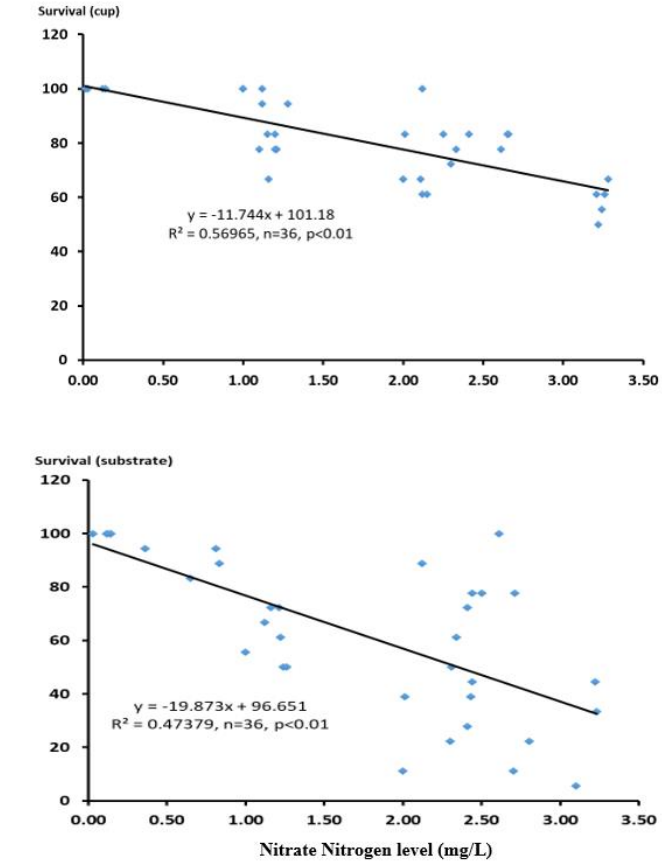

Figure 9. Survival of crablets (%) at Nitrate (NO_3) levels (mg/L) during nursing with Cup (top) and Substrate (down) methods.

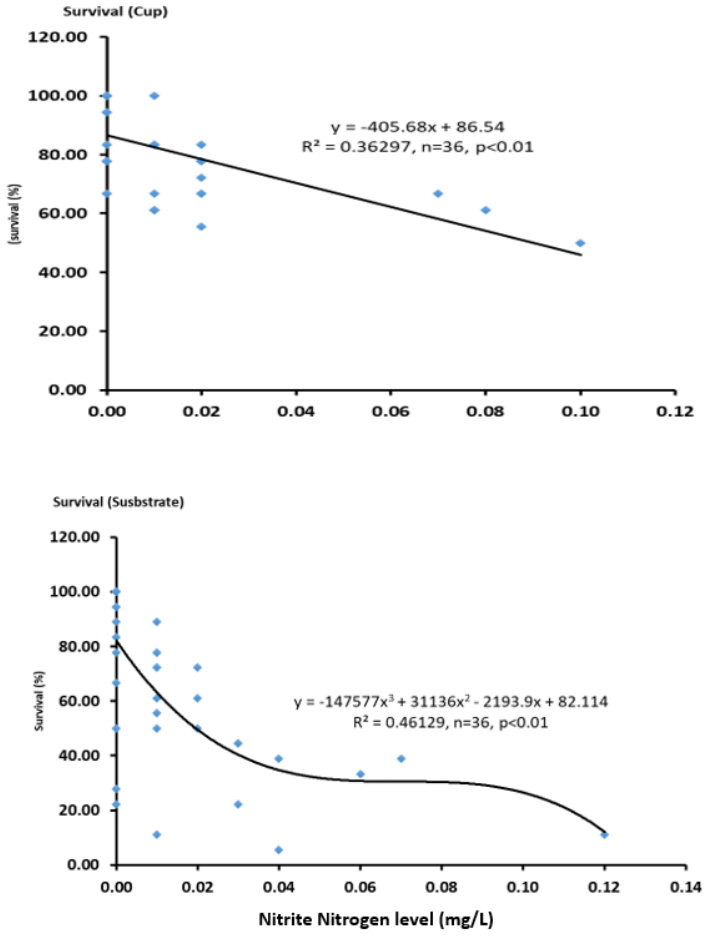

Figure 10. Survival of crablets (%) at Nitrite (NO_2) levels (mg/L) during nursing with Cup (top) and Substrate (down) methods.

Hewitt and Duncan (2001) in the survival of *Marsupenaeus japonicus* juveniles due to cause of physiological damages, such as failure of enzyme systems, denaturation of proteins, respiratory stress or deterioration in membrane structures. High temperature normally increases metabolic rate and ammonia excretion causing toxicity (Magallón, Magallón, Servin, Portillo & Moreno, 2006; Portillo, Portillo, Casillas, Servín & Magallón, 2012). As the main objective for farming or stocking is to increase the number of crablets, survival is more important than growth during the nursing period. Therefore, low temperatures such as 25-26°C can be suggested based on the present study. Similarly, salinity has significant effect on survival and growth of crablets. When compared to 29 ppt and 30 ppt data, 1.67 times higher survival at 30 ppt salinity as compared to the survival at 29 ppt indicates that a minimum range of salinity might be resulted in poor effect of production. According to Romano and Zheng (2012), a minor increase in salinity level may weaken decapods ability to tolerate high ammonia stressor that interferes with the osmoregulatory system. A wide fluctuation in temperature and salinity may cause a profound effect on survival. When the Cup method was used, dissolved oxygen had a positive correlation with crablet survival between 5-6 ppm. However, in the Substrate method, survival did not continue to increase, showing a peak at 5.8 mg/L. This indicates that aeration was needed for Cup method, and less important when using Substrate method. It might be so because when the crablets were with the substrate, they could still come out and freely swim around which was easier to take oxygen for their survival and vital functions without the additional supply artificially. It was not possible to determine the optimum level of DO requirement from the data recorded. As pH control ammonia, Nitrite, and Nitrate levels, it was very important in maintaining the water quality. In this trial, when pH was higher than 8 the survival was found to decrease. Therefore, pH was likely playing an important role in ammonia production and it could be a required factor for higher survival. In addition, pH also plays vital role in culture system which can directly affect the organism's well-being in terms of metabolism and physiological processes. A slight change in pH would give negative effects on crabs because the changes often take place due to residual feed and excretion product of organisms (Talpur & Ikhwanuddin, 2012). In addition to pH, Nitrite (NO_2) and Nitrate (NO_3) had clear evidence of having negative association with survival, which is obvious. However, ammonia nitrogen was not associated with survival or growth of crablets.

Conclusions

Nursing of crablets using individual Cup method improves survival of crablets by about three folds over Substrate method. However, it is time consuming and labour intensive but it warrants further research to explore avenues to develop more practical nursing methods. It is also clear that optimum water quality parameters are also very important to achieve high survival. The water temperature of 25°C, salinity 30 ppt, pH below 8, DO approx. 6 mg/L, NO_2 below 0.1 mg/L and NO_3 below 1.0 mg/L are recommended based on the present findings. However, more comprehensive research needs to be done to further determine these parameters and also ammonia levels. Despite the expectation, supplementation of astaxanthin did not show improvement in survival and growth of crablets. More research should be done.

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