Effect of Feeding Frequency on Growth, Survival, Water and Bottom Soil Qualities in Blue Swimming Crab (*Portunus pelagicus*) Pond Culture Systems

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ABSTRACT

The objective of this study was to improve blue swimming crab, *Portunus pelagicus*, rearing techniques by investigating the feeding frequency on crab survival and changing the properties of the crab pond bottom soil. Crabs were reared under simulated earthen pond conditions in 1.5×2.5×1.2 m^2 concrete ponds for 120 days, at a density of 5 crabs/m^2. During the culture period of 1–30, 31–60 and 61–120 days, crabs were fed with shrimp feed (STARTEQC™) No. 2, No. 4S and No. 4 at 30%, 5% and 3% of body weight per day, respectively. Results showed that the survival rate of crabs fed 2 times (9.00 AM and 3.00 PM; control) and 4 times (7.00 AM, 1.00 PM, 7.00 PM and 11.00 PM) per day were not significantly different, and were higher than those fed 6 times (7.00 AM, 11.00 AM, 3.00 PM, 7.00 PM, 11.00 PM and 3.00 AM) per day. This was especially true during the 60 to 105 days culture period. Feeding frequencies of 2, 4 and 6 times per day did not affect water quality, average daily growth and feed conversion ratio of the crabs. In addition, feeding frequencies of 2, 4 and 6 times per day did not effect changes in organic matter concentration, soil pH and sulfide concentration in the crab pond bottom soil. However, ammonia concentration in the pond bottom soil with the feeding frequency of 6 times per day was higher than with those fed 2 and 4 times per day, especially during 30 to 90 days of culture. This study recommends that a feeding frequency of 2 or 4 times per day is optimal for blue swimming crab culture and does not affect the quality of water and soil in the crab pond.

Keywords: *Portunus pelagicus*, feeding frequency, growth, survival, water and soil qualities

INTRODUCTION

The blue swimming crab, *Portunus pelagicus*, a commercially important species, is distributed throughout the coastal waters of tropical regions of the western Indian Ocean and the Eastern Pacific (FAO, 2009). In Thailand, blue swimming crabs are caught in the Andaman Sea and the Gulf of Thailand for direct consumption and for use as raw material in the food processing industry. Annual exportation of fresh, chilled, or

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frozen crabs to the USA, Japan, Taiwan, and other countries, is a multi-million dollar revenue business for Thailand. However, the harvest of crabs from capture fisheries has shown a decreasing trend due to overfishing and/or changes in coastal and marine environments. For example, quantities of blue swimming crab harvested from the seas of Thailand dropped from 24,200 t in 2007 to 19,216 t in 2013 (Department of Fisheries, 2015). Therefore, the culture of crabs is believed to be one way to increase productivity without placing pressure on wild stock and increase job stability for those involved in commercial crab culture.

Currently, many countries are actively involved in blue swimming crab culture and research, e.g. Australia, India, Indonesia, Malaysia and the Philippines (Josileen and Menon, 2005; Romano and Zeng, 2008). In Thailand, blue swimming crab culture methods have been developed for breeding, nursing, and rearing to gain higher productivity and survival rates. The method of rearing crab broodstock in earthen ponds is also well developed (Oniam and Arkronrat, 2013). However, in most of the studies to date, blue swimming crab production has been subjected to experimentation. Rearing technologies for crab production have not been established on a commercial scale and rearing alone cannot maintain a farmer´s income because of low productivity (Oniam *et al*., 2011a).

Factors that contribute to low survival rate of crab rearing were identified as cannibalism (Marshall *et al*., 2005; Oniam *et al*., 2011b), nutritional quality of feed (Chaiyawat *et al*., 2008; Soundarapandian and Dominic Arul Raja, 2008) and pond bottom soil quality (Oniam, 2011), among others. Oniam (2011) also reported that the factors that contribute to lower productivity of commercial crab culture were cannibalism and soil quality, especially after 90 days of culture. This is the period when crab mortality us caused more by the quality of pond bottom than cannibalism. To improve income from crabs, optimal rearing conditions must be determined. Thus, the objectives of this study were to improve rearing techniques by investigating the effect of feeding frequency on growth, survival, water quality, and changes in properties of bottom soil quality in blue swimming crab production. The knowledge gained from the research will be useful for crab production and in crab farming development, which are important factors related to farmers' future job stability.

MATERIALS AND METHODS

Source of experimental crabs

Broodstock of the blue swimming crab, *P. pelagicus*, were caught using crab traps used by small-scale fishermen in the coastal area of Prachuap Khiri Khan Bay, Prachuap Khiri Khan province, Thailand. The female crabs with dark grey eggs were placed in 200 L fiber tanks to allow the release of eggs for hatching. They were not fed during this period. The newly hatched crab larvae were transferred to 3,000 L concrete tanks for nursing at a density of 100 crabs/L. Newly hatched larvae were initially fed with rotifers (*Branchionus* sp.) and *Chaetoceros* sp. From the zoea II stage onwards they were fed with *Artemia* nauplii

(Arkronrat and Oniam, 2012). Upon larval metamorphosis to the first crab stage they were fed with shrimp feed No. 1 (STARTEQC™, pellet size about 0.40–0.42 mm, 38% protein) until the experiment commenced.

Experimental design and set-up

The experiment consisted of three feeding frequencies as treatments, namely, 2 times (9.00 AM and 3.00 PM (control)); 4 times (7.00 AM, 1.00 PM, 7.00 PM, and 11.00 PM); and 6 times (7.00 AM, 11.00 AM, 3.00 PM, 7.00 PM, 11.00 PM, and 3.00 AM), with equal amount of feed and three replicates per treatment.

Crabs with a carapace width of 1.5–2.0 cm (about 45 days old) from the concrete nursing tanks were transferred to simulated earthen pond conditions in 1.5×2.5×1.2 m^2 concrete ponds, at a density of 5 crabs/m^2 (18 crabs/pond). Crabs were fed with shrimp feed according to Oniam *et al*. (2012). During the first 30 days of the 120-day experimental culture period, crabs were fed with shrimp feed No. 2 (STARTEQC™, pellet size of 0.8–1 mm, 38% protein) at 30% of body weight per day. At 31 to 60 days of culture, crabs were fed with shrimp feed No. 4S (STARTEQC™, pellet size of 3.5 mm, 38% protein) at 5% of body weight per day, then at 61 to 120 days of culture, crabs were fed with shrimp feed No. 4 at 3% of body weight per day. The total number of crabs from each treatment was used to evaluate crab growth and their survival rate every 15 days. For each crab, the carapace width (cm) and body weight (g) were recorded. The carapace width was measured between the tips of the epibranchial spines using vernier calipers. The body weight was measured by digital weighing. At the end of the experiment, the average daily growth (ADG) and feed conversion ratio (FCR) were calculated.

During the experiment, 50% of the water volume was changed once a week. Water quality was analyzed twice a week. Salinity was measured using a refractometer (Primatech; Jakarta, Indonesia), pH using a portable pH meter (pH 11; Cyber Scan; Nijkerk, the Netherlands), temperature and dissolved oxygen concentration (DO) using an oxygen meter (550A; YSI Inc,; Yellow Springs, OH, USA), and total ammonia, nitrite and alkalinity using the indophenol blue method, the colorimetric method, and the titration method, respectively (American Public Health Association *et al*., 2005).

In addition, the bottom soil quality was analyzed once a month by collecting bottom soil samples from two places in each simulated earthen pond using a 5 cm diameter, clear plastic, core liner tube (Wildlife Supply Company, Buffalo, New York, USA). The upper 5 cm segment of each core was removed as described by Munsiri *et al*., (1995), and two core segments from each pond were combined to provide a composite sample for analysis. Tests were undertaken for organic matter concentration using the ignition loss method (Chareonpanich and Seurungreong, 1997), for soil pH using the method described by Thunjai *et al*. (2001), for total ammonia concentration using the method described by Chuan and Sugahara (1984), and for sulfide concentration using the acid voltaic sulfides test described by Allen *et al*. (1993).

Statistical analysis

Data on the growth, survival, ADG, and FCR, including water and bottom soil qualities 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. Data were analyzed using the IBM SPSS Statistics program.

RESULTS AND DISCUSSION

The study of blue swimming crab (*P. pelagicus*) grown under simulated earthen pond conditions was conducted for 120 days. Optimum environmental conditions were maintained during the experimental culture period, with mean values presented in Table 1. Average values of water quality samples from the crab rearing ponds at different feeding frequencies were not significantly different ($P>0.05$; Table 1). These parameters have never affected the growth and survival of this crab species (Lignot *et al*., 2000; Romano and Zeng, 2006; 2007; Maheswarudu *et al*., 2008; Oniam *et al*., 2011b; Oniam and Arkronrat, 2013).

The averages of the carapace width, body weight and survival rate of crabs are shown in Table 2. Survival rates of crabs fed 2 and 4 times per day were not significantly different ($P>0.05$) and were higher than those fed 6 times per day, especially during 60 to 105 days of culture ($P<0.05$). The ADG and FCR of crabs fed 2 times (0.65±0.13 g/day and 3.90±0.18, respectively), 4 times (0.55±0.02 g/day and 3.97±0.44, respectively), and 6 times (0.50±0.04 g/day and 5.08±0.94, respectively) per day were also not significantly different (P>0.05; Figure 1).

Table 1. Water quality parameters during experimentation with blue swimming crab, *P. pelagicus*, reared under simulated earthen pond conditions at different feeding frequencies (mean±SD).

Parameter	Feeding frequency (per day)			*P*-value
	2 times	4 times	6 times	
Water temperature (ºC)				
AM	28.1±0.0	28.1±0.0	28.2±0.0	0.414
PM	30.6±0.0	30.6±0.0	30.6±0.0	0.911
Dissolved oxygen (mg/L)				
AM	4.08±0.11	4.08±0.35	4.06±0.77	0.930
PM	6.00±0.11	6.06±0.24	6.02±0.13	0.918
pH				
AM	7.62±0.41	7.64±0.25	7.63±0.01	0.700
PM	7.71±0.04	7.71±0.25	7.73±0.11	0.706
Salinity (ppt)	32.7±0.1	32.6±0.0	32.6±0.0	0.824
Total ammonia (mg-N/L)	0.09±0.03	0.05±0.01	0.07±0.02	0.258
Nitrite (mg-N/L)	0.03±0.01	0.08±0.05	0.03±0.01	0.210
Alkalinity (mg/L as $CaCO_3$)	125.5±4.5	121.0±4.7	125.2±2.4	0.381

Table 2. Carapace width (CW), body weight (BW) and survival rate (SV) of blue swimming crab, *P. pelagicus*, raised under simulated earthen pond conditions at different feeding frequencies (mean±SD).

Culture period (days)	Feeding frequency (per day)								
	2 times			4 times			6 times		
	CW (cm)	BW (g)	SV (%)	CW (cm)	BW (g)	SV (%)	CW (cm)	BW (g)	SV (%)
Initial	$1.82±0.22^{a}$	$0.52±0.16^{a}$	$100±0.00^{a}$	$1.82±0.22^{a}$	$0.52±0.16^{a}$	$100±0.00^{a}$	$1.82±0.22^{a}$	$0.52±0.16^{a}$	$100±0.00^{a}$
15	$3.33±0.49^{a}$	$8.33±1.34^{a}$	$87.03±8.45^{a}$	$3.10±0.36^{a}$	$7.36±0.15^{a}$	$83.33±5.55^{a}$	$3.60±0.34^{a}$	$9.10±1.34^{a}$	$87.03±3.23^{a}$
30	$4.43±0.28^{a}$	$20.93±1.98^{a}$	$74.10±6.40^{a}$	$4.36±0.15^{a}$	$18.90±2.12^{a}$	$72.23±9.58^{a}$	$4.53±0.32^{a}$	$17.76±1.10^{a}$	$70.40±6.40^{a}$
45	$7.50±0.36^{a}$	$25.83±2.46^{a}$	$57.43±8.49^{a}$	$7.33±0.15^{a}$	$27.50±1.70^{a}$	$68.50±6.40^{a}$	$7.00±0.20^{a}$	$24.43±1.06^{a}$	$59.26±11.54^{a}$
60	$7.53±0.05^{a}$	$33.10±1.12^{a}$	$51.86±6.46^{ab}$	$7.40±0.10^{a}$	$35.43±3.38^{a}$	$61.13±5.55^{a}$	$7.30±0.20^{a}$	$30.20±2.59^{a}$	$48.13±3.23^{b}$
75	$8.20±0.20^{a}$	$43.46±3.49^{a}$	$50.00±5.60^{ab}$	$8.20±0.17^{a}$	$43.56±2.36^{a}$	$55.60±0.00^{a}$	$7.60±0.10^{b}$	$31.73±2.01^{b}$	$44.40±0.00^{b}$
90	$8.90±0.50^{a}$	$55.70±8.22^{a}$	$46.26±3.23^{a}$	$8.70±0.26^{a}$	$49.00±4.25^{a}$	$50.00±5.60^{a}$	$8.46±0.11^{a}$	$45.53±2.11^{a}$	$31.46±3.17^{b}$
105	$9.13±0.49^{a}$	$62.06±9.99^{a}$	$42.56±3.17^{a}$	$9.00±0.65^{a}$	$58.43±6.65^{a}$	$40.73±8.49^{a}$	$8.70±0.36^{a}$	$48.13±3.96^{a}$	$29.63±3.17^{b}$
120	$10.40±0.20^{a}$	$79.03±15.98^{a}$	$31.46±11.54^{a}$	$9.56±0.55^{b}$	$67.60±2.61^{a}$	$33.33±5.55^{a}$	$9.26±0.11^{b}$	$60.10±5.36^{a}$	$25.93±3.23^{a}$

Note : Data in the same row and same parameter for different feeding frequencies with different letters are significantly different ($P<0.05$).

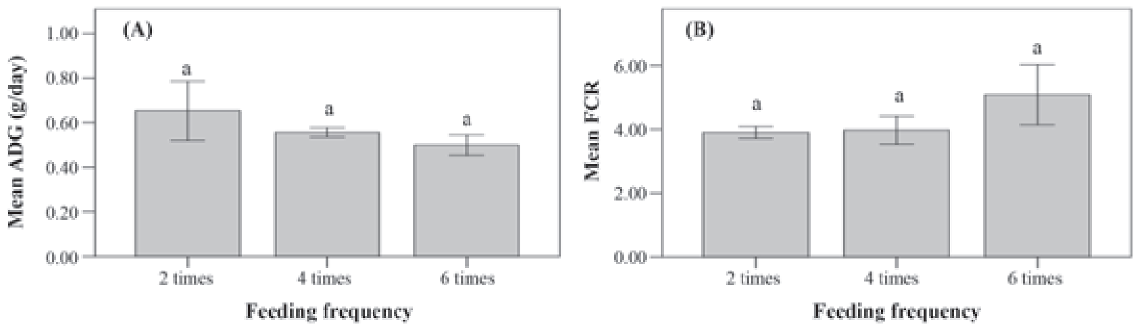

Figure 1. Average daily growth (A) and feed conversion ratio (B) of blue swimming crab, *P. pelagicus*, raised under simulated earthen pond conditions at different feeding frequencies. Error bars indicate mean±SD.

The average organic matter, soil pH, ammonia, and sulfide concentrations in the bottom soil with different feeding frequencies are shown in Figure 2. The results showed that feeding frequencies did not affect organic matter concentration, soil pH, and sulfide concentration in crab pond bottom soils (P>0.05; Figure 2). However, ammonia concentration in the crab pond bottom soil with a feeding frequency of 6 times per day was higher than those with feeding frequencies of 2 and 4 times per day, especially during 30 to 90 days of culture period (P<0.05; Figure 2).

Feeding frequency was an important factor to be considered as it can affect growth and survival of crabs, with feeding at the optimum frequency resulting in tremendous savings in feed cost (Robertson *et al*., 1993; Nair and Sridhar, 2003). In the current experiment, crab survival rate from 60 to 105 days with a feeding frequency of 6 times per day was affected resulting in an average survival rate that was significantly lower (decreased from 48.13 to 29.63%; Table 2) than those with feeding frequencies of 2 and 4 times per day (decreased from 51.86 to 42.56%, and from 61.13 to 40.73%, respectively; Table 2). The crab death rate may have been caused by cannibalism due to limited food availability (increasing to 6 times per day but the same amount of feed). In contrast, in other crab species, a proper feeding frequency and the amount of food given to the reared species were paramount to improve growth and production. For example, Shelley and Lovatelli (2011) reported that the growth and survival of mud crab, *Scylla* spp., were increased as the feeding frequency increased.

The occurrence of cannibalism was usually associated with limited food availability, high population density and limited space (Marshall *et al*., 2005). Cannibalism has been reported to be a serious problem in blue swimming crab, *P. pelagicus*, aquaculture (Oniam and Arkronrat, 2013; Azra and Ikhwanuddin, 2015). Many researchers have reported that *P. pelagicus* in earthen ponds showed low productivity at 90–120 days (decreased from 140.50 to 26.74 kg/1,600 m^2 at harvest) due to a low

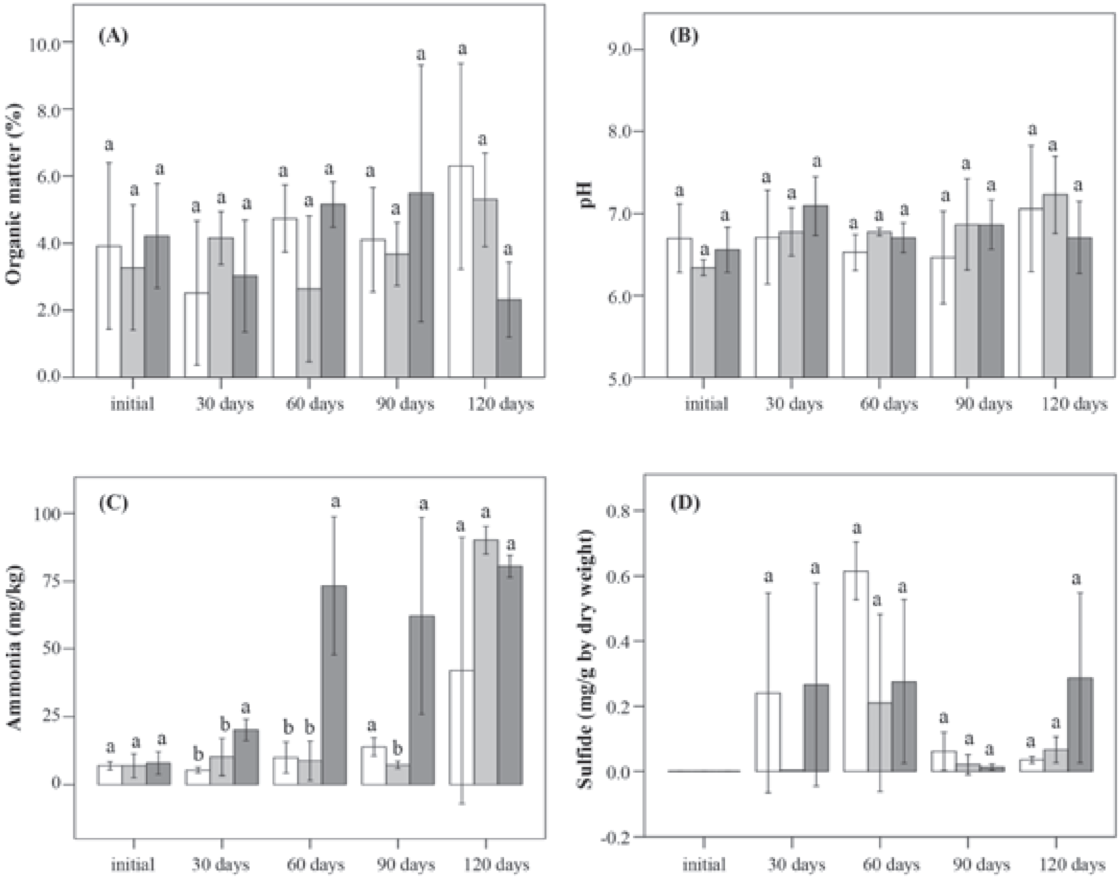

Figure 2. Organic matter (A), pH (B), ammonia (C), and sulfide (D) in bottom soil of blue swimming crab, *P. pelagicus*, rearing ponds at different feeding frequencies: 2 times (□), 4 times (■) and 6 times (■) per day. Different lower case letters above each bar indicate a significant difference at $P<0.05$. Error bars indicate mean±SD.

survival rate (decreased from 59.59 to 2.97%) (Kedmuean *et al*., 2004; Thepphanich and Chumworrathayee, 2005; Thepphanich *et al*., 2008). However, the mortality rate of this crab in grow-out ponds due to cannibalism decreases as the crab's age increases. Furthermore, after 90 days of culture, other factors contributed more to the mortality of the crabs (Oniam *et al*., 2011b). Oniam (2011) also reported that the factors that contribute to lower productivity of this crab culture were cannibalism and soil quality, especially after 90 days of culture. This is the period when the pond bottom soil quality causes crab mortality more than does cannibalism, i.e. the changing properties due to ammonia concentration and soil pH in the pond bottom soil affect the productivity of crabs. The results of this study showed that the crab pond bottom soil at a feeding frequency of 2, 4 and 6 times per day had organic matter concentrations in the range of 1.13–8.75 (4.32±1.35), 1.14–6.78 (3.81±1.00), and 1.49–9.11 (4.04±1.36)%, respectively, soil pH in the range of 6.20–7.68 (6.69±0.23), 6.23–7.67 (6.79±0.31), and 6.21–7.50 (6.78±0.21), respectively, and sulfide concentration in the range of 0.00–0.67 (0.19±0.25), 0.00–0.52 (0.06±0.09), and 0.00–0.62 (0.16±0.15) mg/g by dry weight, respectively. These parameters did not affect the growth and survival of blue swimming crab (Oniam, 2011). Ammonia concentrations in the crab pond bottom soil at feeding frequencies of 2, 4 and 6 times per day ranged from 4.49 to 98.40 (15.57±23.37), 2.23 to 94.87 (24.59±36.57), and 3.20 to 93.43 (48.74±32.70) mg/kg, respectively. The average ammonia concentration in the crab pond bottom soil with the feeding frequency of 6 times per day was higher than those at feeding frequencies of 2 and 4 times per day, especially during 30 to 90 days of culture. From 60 to 105 days of culture, crab survival rates with a feeding frequency of 2 times (42.56–51.86%) and 4 times (40.73–61.13%) per day were higher than those with feeding frequency of 6 times (29.63–48.13%) per day (Table 2). The crab death rate may have been caused by the effect of ammonia in the soil. Oniam (2011) reported that blue swimming crab reared in pond soil with high ammonia levels (24.62±20.36 mg/kg) had lower productivity (0.06 kg/m^2) and higher FCR (4.01±0.11) compared to those in pond soil with lower ammonia levels (6.72±4.84 mg/kg) which had productivity at 0.11 kg/m^2 and FCR at 3.41±0.23. He further reported that ammonia concentration in the crab pond soil was an important factor affecting crab growth and survival. Thus, the properties of the crab pond bottom soil are important parameters influencing crab production.

Pond bottom soil quality is an important factor in aquaculture, with the water quality in aquaculture ponds influenced by the exchange of substances between soil and water. The four most important features of soils with regard to aquaculture production are texture, organic matter concentration, pH, and nutrient concentration (Boyd, 1995). In this study, ammonia concentration in the crab pond bottom soil was positively related to increased culture period and this affected productivity (Figure 3). Similar results for other crustacean species have been reported, e.g., marine shrimp culture (Kumar *et al*., 2012). Boyd (1995) stated that fertilizer and formulated feed were important sources of nitrogen in ponds. Among the three major nitrogenous compounds (ammonia, nitrite and nitrate), ammonia-N was generally the most toxic to aquatic animals. Romano and Zeng (2010) reported that the 96-h LC_{50} values of ammonia-N for *P. pelagicus* juveniles at salinity levels of 15, 30, and 45 ppt were 37.8–48.2, 60.1–70.9, and 69.4–80.7 mg/L, respectively, while the 96-h LC_{50} values of NH_3 was 2.5–3.3, 4.0–4.7 and 4.3–5.0 mg/L, respectively, and that the ammonia-N tolerance of *P. pelagicus* significantly decreases with decreasing salinity. In contrast, Rebelo *et al*. (2000) reported that ammonia-N toxicity to the estuarine crab, *Neohelice* (*Chagmanathus*) *granulata*, increased at low salinities. This report supports the current experiment where the crab mortality rate at 60 days onwards was caused by ammonia concentration in the bottom soil, especially for feeding frequency of 6 times per day (62.2–80.3 mg/kg ammonia). However, studies on the bottom soil quality in crab ponds are very limited, and improving or controlling pond bottom soil quality to increase blue swimming crab survival are interesting points for future study.

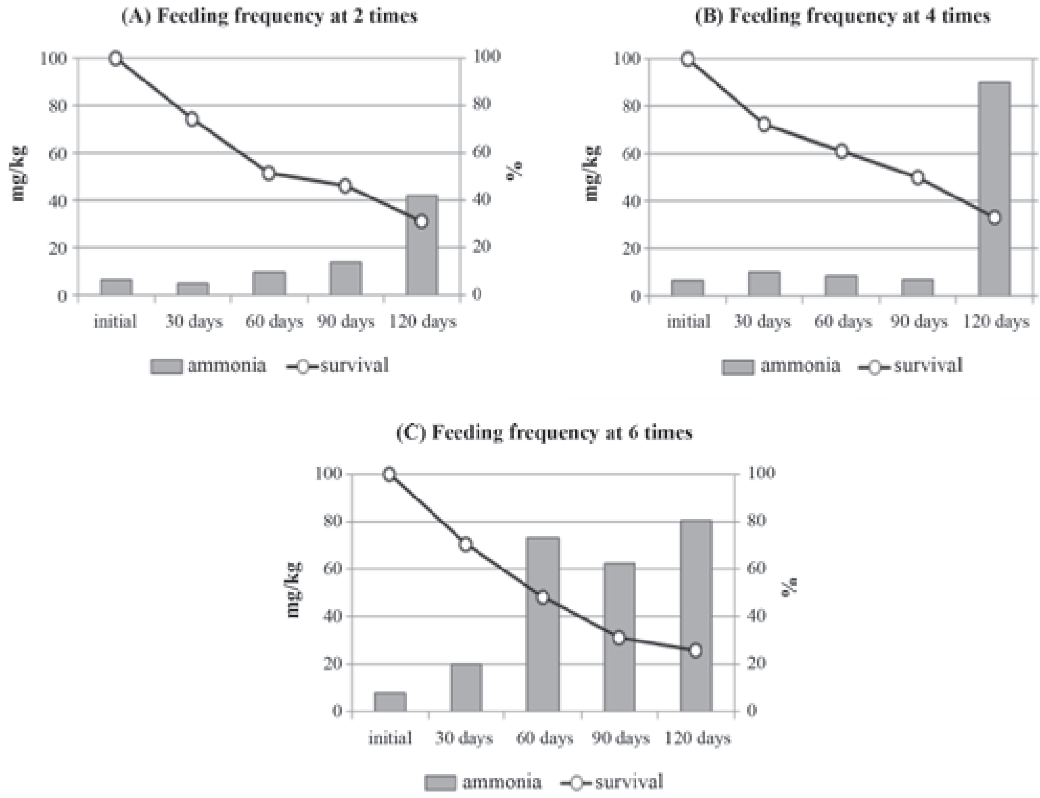

Figure 3. Average ammonia concentrations in pond bottom soil and survival rate of blue swimming crab, *P. pelagicus*, reared at different feeding frequencies.

CONCLUSION

This study demonstrated that feeding frequencies affected the survival of blue swimming crab, *Portunus pelagicus*. Crab survival rates with feeding frequencies of 2 and 4 times per day were higher than those with a feeding frequency of 6 times per day, especially during 60 to 105 days of culture. However, feeding frequency did not affect average daily growth and feed conversion ratio of crabs. In addition, feeding frequencies of 2, 4, and 6 times per day did not affect water quality based on organic matter concentration, soil pH, and sulfide concentration in the crab pond bottom soil. On the other hand, the ammonia concentration in pond soils with feeding frequency of 6 times per day was higher than those with feeding frequencies of 2 and 4 times per day, especially during 30 to 90 days of culture. These factors affected crab production. The development of approaches to improve crab production and reduce crab mortality are interesting and useful topics for future study, i.e. research on shelter quality (number, size, and type of shelter) to reduce crab mortality caused by cannibalism, and investigating ways to improve pond bottom soil quality to increase crab survival.

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