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Effects of Ionic Concentrations on Survival and Growth of *Penaeus monodon* Reared in Low-Salinity Waters

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ABSTRACT

A comparative study was made of two black tiger shrimp (*Penaeus monodon*) farms operating under low-salinity water conditions located in the same vicinity. Each farm had 12 earthen ponds with an area of 4 rai (6,400 m^2). Farm 1 achieved normal production over the past six years, while farm 2 operated for only two years had low production in each growout cycle. Postlarvae (PL) were stocked at a rate of 80,000 shrimp/rai (50 shrimp/m^2). After harvesting, farm 1 had an average production of 899 kg/rai, shrimp body weight of 14.69 g, growth rate of 0.129 g/day and feed conversion rate (FCR) of 1.38. These results were significantly different from farm 2 which had an average production of 560 kg/rai, body weight of 11.66 g, growth rate of 0.096 g/day and FCR of 1.91 ($P<0.05$). The average survival rate in farm 1 was 70.8±12.45 % compared with 60.1±17.1 % in farm 2. However, there was no significant difference ($P>0.05$). Analysis of the six major ions (Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^- and SO_4^{2-}) one day before stocking PL found that the ion concentrations in both farms were approximately the same and the salinity five days after stocking was approximately 5 parts per thousand (ppt). At day 30, the ion concentrations in the low -production farm started to decrease lower than those of the normal-production farm, especially Na^+ and Mg^{2+}. Sixty days after stocking, Na^+ and K^+ concentrations from the low-production farm were significantly lower than the normal-production farm ($P<0.05$). Two probable causes were that the seepage in the low-production farm was higher due to a lower percentage of clay particles in the soil and that the level of the pond bottom was higher than the freshwater canal which surrounded the ponds. More freshwater was added to replace the seepage in the low-production farm than in the normal-production farm, so that salinity was diluted more quickly and affected all major ion concentrations. In particular,

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Na^+ and K^+ concentrations in the low-production farm were significantly lower than in the normal-production farm ($P<0.05$). Based on the results from this study it could be concluded that in order to achieve normal survival and growth of black tiger shrimp reared under low -salinity waters, the salinity for stocking should not be lower than 5 ppt and the ionic profiles should be similar to the seawater. During the first 30 days, salinity should be maintained or not allowed to drop quickly. The minimum levels of Na^+, Mg^{2+} and K^+ during the culture period should be maintained not less than the concentration of seawater at 3 ppt.

Key words : ionic concentrations, *Penaeus monodon*, low-salinity waters

INTRODUCTION

Due to frequent viral and bacterial disease outbreaks in intensively farm-reared black tiger shrimp (*Penaeus monodon*) in the coastal cultivated areas, inland shrimp farming under low-salinity conditions expanded rapidly throughout Thailand during the 1990s. Notably in 1998, inland low-salinity shrimp farming represented as much as 40 percent of Thailand's total production (Limsuwan, 2000a). This is because shrimp farms that use water with a low salinity are less prone to disease problems than those using normal seawater (Limsuwan, 2000b). Inland shrimp farming practices are generally similar to those used in typical coastal operations. The primary difference is that coastal farms use natural saline water (15-30 ppt) for culturing, while shrimp farms using low-salinity water obtain water from two sources, either from brackish water streams or rivers, or brine solution (100-200 ppt salinity). Shrimp farmers who use brine solution dilute it with freshwater to a salinity level of 3-5 ppt for stocking postlarvae (PL) and then add more freshwater during the culture period so that the salinity level is down to 1-2 ppt by the time the shrimp are harvested (Limsuwan, 2000b; Szuster and Flakerty, 2002; Limsuwan and Chanratchakool, 2004).

Inland shrimp farming is now carried out in both in saline coastal wetlands and arid regions far from the sea (Jiang and Gong, 2002), in countries such as the U.S.A., Ecuador and in several countries in Latin America (Smith *et al.*, 1990; Rosenberry, 2000; Nunes and Lopez, 2001; Treece, 2002). However, because of variations in water and soil quality, some farmers have had problems rearing shrimp while others have been more successful. Compared with seawater, the ion profile has wide fluctuations, and the rule of constancy of composition of seawater does not apply to some inland farms (Forsberg and Neill, 1997). Furthermore, the ionic composition and salinity of the water in different locations can vary markedly; the natural saline water resources in many inland places cannot be used directly in shrimp culture (Boyd, 2002; Saoud *et al.*, 2003). Davis *et al.* (2002) stated that the ionic composition of saline water appears to be more important than salinity with regard to its effect on shrimp survival and growth. Boyd and Thunjai (2003) analyzed the concentrations of major ions of water samples from 40 inland shrimp farms in China, Ecuador, Thailand and U.S.A. and found that potassium deficiency was a common phenomenon. Several researchers have been working to identify the reasons for differences in survival and growth among farms or the necessary minimum ion requirements in the water for marine shrimp culture. (McGraw *et al.*, 2002; McGraw and Scapa, 2003; Davis *et al.*, 2004)

The purpose of the present study was to analyze ionic composition between two low-salinity farms that were located in close geographical proximity to investigate the ion profile differences on a farm with normal growth and survival compared with a farm with slow growth and a low survival. The results from this study should reveal to which factors farmers should pay special attention for preparing and maintaining water quality when rearing shrimp in low-salinity conditions.

MATERIALS AND METHODS

This study was carried out in two black tiger shrimp farms using a closed water recirculation system located in close proximity to each other in Bangphae district, Ratchaburi province. Each farm had 12 earthen ponds with an area of 4 rai (6,400 m^2) with the reservoir pond 30 percent of culture areas. Over the past six years, farm 1 achieved normal harvests of 700-1,200 kg/rai (1rai=1,600 m^2) in each 120-day growout cycle. Farm 2 had been operated only two years with an average harvest of 400-600 kg/rai in each 120-day culture cycle, despite using the same source of shrimp larvae and management. Before stocking, soil samples at the bottom of each pond were collected and analyzed for the soil texture, organic matter, phosphorus, potassium, calcium and magnesium at the Department of Soil Science, Faculty of Agriculture, Kasetsart University, Bangkok. To start the shrimp rearing process, freshwater from the reservoir pond was pumped into the growout ponds at a depth of 70-80 cm. Brine solution

(100-200 ppt salinity) from a salt farm was placed in a plastic nursery enclosure (150 m^2) until the salinity was adjusted down to 8-10 ppt. The water outside the plastic nursery trough in the pond was diluted to 3-5 ppt.

The PL stage 15 (PL15) used in this study were produced from the same hatchery and diagnosed to be free from white spot syndrome virus (WSSV) by the polymerase chain reaction (PCR) assay. The PL15 were stocked into the plastic nursery enclosure at a rate of 80,000 shrimp per rai (50 shrimp/m^2). Salinity was gradually adjusted to the outside salinity during three days of nursing before all shrimp were released from the plastic nursing enclosure. Commercial feed pellets for black tiger shrimp were fed four times daily, and the feeding program was adjusted according to the method by Limsuwan (2000b). During the first 90 days of the growout period, water was not changed but some water from the reservoir pond was added to replace water lost due to evaporation or soil seepage until the water level reached a depth of 1.3-1.4 m. Water was changed if the transparency was too low due to the accumulation of suspended solids or a heavy phytoplankton bloom. All water that was pumped out of the rearing ponds was discharged back into the reservoirs. None was released outside the farms.

<u>Major ion analysis</u>

Samples of water from each pond were collected one day before the shrimp were stocked into the plastic nursery confinement and then into the growout ponds at days 5, 30, 60, 90 and one day before harvesting. Water samples were analyzed for the concentrations of calcium (Ca^{2+}), magnesium (Mg^{2+}), sodium (Na^{+}) and potassium (K^{+}) ion using the atomic absorption spectrophotometer (Hitachi 170-30, Japan) while chloride (Cl^{-}), bicarbonate (HCO_3^{-}) and sulfate (SO_4^{2-}) ion concentrations were analyzed using the titration method (UNEP. GEMS, 1994). Salinity was measured with a salinometer (YSI 30/10 FT, U.S.A.).

<u>Statistical analysis</u>

At the end of culture period, shrimp were harvested and the average yield, body weight of the shrimp, percent survival and feed conversion rates (FCR) of each pond were recorded. All the data from both farms were statistically compared using t-test (Steel and Torrie, 1980)

RESULTS AND DISCUSSION

<u>Production, survival, growth and FCR</u>

The average production, survival and growth data of the shrimp from both farms are presented in Table 1. There were significant differences in production, body weight, growth and FCR between farm 1 and farm 2 ($P<0.05$). No significant differences in survival between farm 1 and farm 2 were found. At the end of growout period, the final body weights of farm 1 (which had normal growth) was 14.69 g or a growth rate of 0.129 g/d, significantly higher than the average weight of farm 2 which was 11.60 g or a growth rate of 0.096 g/d ($P<0.05$).

The average survival of shrimp from farm 1 was 70.8±12.45 percent, while it was only 60.1±17.1 percent in farm 2. But there was no significant difference because of the variation among the 12 ponds in each farm. In farm 1, the survival ranged between 46.3 to 94.7 percent, while in farm 2, survival ranged from 30.2 to 78.4 percent. The average of 10 percent difference in survival and better growth per day of shrimp in farm 1 resulted in differences in the production. The average production in farm 1 was 899 kg/rai (1,600 m^2) which was significantly higher than that from farm 2 of 560 kg/rai ($P<0.05$). Higher production and better growth of shrimp in farm 1 resulted in a lower FCR than farm 2 ($P<0.05$).

Table 1 Production of black tiger shrimp from farm 1 and 2

Parameter	Farm 1	Farm 2
Culture period (days)	115.58 ± 7.87^{a}	121.42 ± 3.7^{b}
Average weight (g)	14.69 ± 2.43^{a}	11.66 ± 2.41^{b}
Production (kg/1,600m^2)	899.08 ± 238.78^{a}	560.33 ± 68.46^{b}
Weight gain/day (g)	0.129 ± 0.024^{a}	0.096 ± 0.021^{b}
Survival rate (%)	70.8 ± 12.45^{a}	60.1 ± 17.1^{a}
Feed conversion rate (FCR)	1.38 ± 0.13^{a}	1.91 ± 0.19^{b}

The different alphabets in the same row mean significant difference ($P<0.05$).

Soil textures

Analysis of soil textures from both farms are presented in Table 2. The percentage of clay particles in farm 1 was significantly higher than that in farm 2, but the percentages of sand and silt were significantly lower than those in farm 2 ($P<0.05$). The amounts of organic matter, phosphorus, potassium, calcium and magnesium in soil samples from both farms were not significantly different (P>0.05). Both farms had very high amounts of calcium compared with magnesium, approximately a 5 to 1 ratio. The average organic matter in the soil samples from the pond bottom of farm 1 was 2.11±1.66 percent which was in the medium range, while in farm 2 it was only 1.26±0.37 percent. Boyd (1995) stated that the amount of organic matter at a medium level between 1.5 to 2.5 percent was suitable for aquaculture. Organic matter may have some effect on the abundance of natural food which results in better shrimp growth.

Table 2 Soil textures from pond bottom of experimental farm 1 and 2

Parameter	Farm 1	Farm 2
Soil textures (%)		
- Sand	18.08 ± 2.47[a]	20.75 ± 3.194[b]
- Silt	19.33 ± 5.28[a]	25.00 ± 7.21[b]
- Clay	62.33 ± 5.82[a]	54.25 ± 6.48[b]
Organic matter (%)	2.11 ± 1.66[a]	1.26 ± 0.37[a]
Phosphorus (mg/kg)	59.42 ± 33.9[a]	59.00 ± 46.14[a]
Potassium (mg/kg)	255.83 ± 57.75[a]	235.83 ± 85.64[a]
Calcium (mg/kg)	5,925.00 ± 1,023.33[a]	5,600.0 ± 3,001.81[a]
Magnesium (mg/kg)	1,166.67 ± 179.43[a]	1,023.33± 30.20[a]
pH	7.28 ± 0.32[a]	7.28 ± 0.47[a]

The different alphabets in the same row mean significant difference (P<0.05).

Major ion concentrations

The ion concentrations during the culture period are shown in Table 3. The concentration of six major ions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} , Cl^{-} and SO_4^{2-}) in the water inside the plastic nursery enclosure one day before stocking of PL15 were similar in the two farms except for HCO_3^{-} which was higher in farm 1. After three days, the shrimp were released out of the plastic confinement into the rest of the pond. The concentration of every ion and salinity measured at the day 5 dropped at both farms because the water outside the nursery baskets had a lower

Table 3 The average ion concentrations (mg/l) and salinity during the culture period of black tiger shrimp ponds from both farms

Culture period (day)	Ca^{2+} Mean±SD	Mg^{2+} Mean±SD	Na^{+} Mean±SD	K^{+} Mean±SD	Cl^{-} Mean±SD	HCO_2^{-} Mean±SD	SO_4^{2+} Mean±SD	Salinity (ppt) Mean±SD
Nursery baskets (1 day before stocking)								
Farm 1 - Normal-production	136.95 ± 25.71^a	187.16 ± 23.17^a	$1{,}351.08 \pm 18.42^a$	89.38 ± 18.70^a	$4{,}591.48 \pm 1{,}198.37^a$	150.57 ± 19.02 a	973.92 ± 107.71^a	9.5 ± 1.06^a
Farm 2 - Low-production	126.73 ± 19.86^a	190.6 ± 29.48^a	$1{,}371.50 \pm 70.33^a$	96.45 ± 25.37^a	$4{,}916.17 \pm 265.30^a$	134.55 ± 15.04^b	$1{,}013.92 \pm 194.97^a$	9.83 ± 1.75^a
Growout ponds (5 days)								
Farm 1 - Normal-production	111.52 ± 26.71^a	145.66 ± 37.09^a	$1{,}184.00 \pm 203.40^a$	68.65 ± 13.81^a	$3{,}136.5 \pm 789.27^a$	147.81 ± 20.09^a	699.42 ± 148.70^a	5.18 ± 0.79^a
Farm 2 - Low-production	104.00 ± 22.35^a	119.63 ± 28.62^a	$1{,}185.33 \pm 125.76^a$	64.2 ± 19.69^a	$3{,}081.33 \pm 926.28^a$	146.78 ± 20.28^a	682.58 ± 208.84^a	5.30 ± 0.63^a
Growout ponds (30 days)								
Farm 1 - Normal-production	97.18 ± 15.24^a	112.09 ± 42.47^a	895.58 ± 166.23^a	40.68 ± 10.76^a	$2{,}078.58 \pm 361.15^a$	118.59 ± 24.53^a	612.17 ± 127.44 a	3.7 ± 0.68^a
Farm 2 - Low-production	86.79 ± 19.12^a	78.83 ± 17.45^b	701.08 ± 190.89^b	30.54 ± 14.02^a	$1{,}805.67 \pm 410.29^a$	102.33 ± 27.99^a	574.25 ± 127.22^a	3.5 ± 0.61^a
Growout ponds (60 days)								
Farm 1 - Normal-production	77.9 ± 17.21^a	83.62 ± 17.79^a	854.25 ± 114.79^a	30.49 ± 3.36^a	$1{,}732.75 \pm 374.40^a$	113.35 ± 32.72^a	461.3 ± 123.70^a	3.5 ± 0.73^a
Farm 2 - Low-production	71.49 ± 22.08^a	70.35 ± 16.23^a	549.08 ± 103.26^b	25.17 ± 6.20 b	$1{,}531.50 \pm 300.09^a$	115.23 ± 43.37^a	483.77 ± 152.38^a	3.13 ± 0.46^b
Growout ponds (90 days)								
Farm 1 - Normal-production	62.88 ± 12.58^a	71.13 ± 16.23^a	759.64 ± 156.23^a	24.31 ± 4.15^a	$1{,}423.58 \pm 408.87^a$	116.94 ± 19.54^a	287.28 ± 105.64^a	3.3 ± 0.37^a
Farm 2 - Low-production	57.40 ± 19.17^a	60.40 ± 13.14^a	454.57 ± 103.69^b	21.38 ± 5.73^a	$1{,}210.92 \pm 390.14^a$	104.97 ± 21.79^a	338.48 ± 151.47^a	2.2 ± 0.39^b
Before harvest 1 day								
Farm 1 - Normal-production	58.09 ± 9.79^a	67.94 ± 17.30^a	706.37 ± 148.10^a	22.25 ± 4.36^a	$1{,}287.58 \pm 290.10^a$	92.92 ± 7.18^a	317.09 ± 111.29^a	2.9 ± 0.38^a
Farm 2 - Low-production	49.17 ± 13.19^a	57.40 ± 11.68^a	449.52 ± 98.97^b	19.61 ± 4.28^a	$1{,}055.92 \pm 323.25^a$	84.85 ± 13.35^a	287.45 ± 116.52^a	1.37 ± 0.37^b

The different alphabets in the same row mean significant difference ($P<0.05$).

salinity than inside.

After the shrimp had been reared for 30 days, the ion concentrations in the water on farm 2 (which had lower production) started to decrease lower than those on farm 1 (which had normal production), especially the concentrations of Na^{+} and Mg^{2+} which were statistically different ($P<0.05$). At day 60, the Na^{+} and K^{+} concentrations from farm 2 were significantly lower than those of farm 1 ($P<0.05$). Two probable causes were that the seepage in farm 2 was higher than in farm 1 due to the lower percentage of clay particles and that the pond bottom level was higher than the level of the freshwater canal surrounding the ponds, so that the water level in the culture ponds on farm 2 dropped more rapidly than on the farm 1. Due to these two main causes, freshwater was added to refill the ponds so that the salinity was diluted more quickly. This affected the concentration of all major ions. Measurements taken when the shrimp were 60-and 90-days-old and one day before harvesting showed that the ion concentrations and salinity dropped consistently throughout the culture period at both farms but dropped more rapidly in farm 2 (which had a lower production). In particular, the amount of Na^{+} and K^{+} on the low production farm was lower than on the normal production farm to a statistically significant degree ($P<0.05$). Based on the results from this study, Na^{+}, Mg^{2+} and K^{+} concentrations in farm 2 were significantly lower than in farm 1. These differences in the ionic concentration of water from both farms affected the survival and growth of the shrimp.

Boyd *et al.* (2002) suggested that seawater has acceptable proportions of the major ions for marine shrimp culture and any other water source could be evaluated based on the magnitude of the deviation in ionic proportions from those of seawater. In one part per thousand of diluted seawater the concentrations of the various minerals should be as follows: Ca^{2+}, 11.6; Mg^{2+}, 39.1; K^{+}, 10.7; Na^{+}, 304.5; Cl^{-}, 551 and SO_4^{2-}, 78.3 mg/l, respectively (Davis *et al.*, 2004). In general , water is suitable for shrimp culture if the salinity is above 5 ppt; levels of Na^{+}, Cl^{-} and K^{+} are similar to the levels in seawater diluted to the same salinity; it has a high concentration of Ca^{2+} and the alkalinity is more than 75 mg/l (Davis *et al.*, 2004). Ionic profiles of water from both farms in this study had high concentrations of Ca^{2+} but low concentrations of Mg^{2+} and Na^{+} when compared with seawater diluted to the same salinity. High amounts of calcium in the soil may affect the Ca^{2+} concentration in the water or brine solution used for stocking PL with a low concentration of Mg^{2+} and Na^{+}. Levels for other ions such as K^{+}, Cl^{-} and SO_4^{2-} were similar to seawater profiles (Table 3). At day 30, the average salinity in farm 2 was 3.5 ppt but the Mg^{2+} level was 78.83 mg/l which is equal to 2 ppt of seawater, while the Na^{+} concentration was 701.08 mg/l, equal to 2.3 ppt of seawater. The significant decrease in $Mg^{2+}$and Na^{+} concentrations in farm 2 affected shrimp survival during the first 30 days in farm 1.

Most researchers reported on the effects of Mg^{2+} and K^{+} deficiency on shrimp survival and growth. Low levels of Mg^{2+} and K^{+} in saline water were responsible for PL mortalities (Rahman *et al.*, 2005) and suggested that if the salinity is adequate, Ca^{2+}, Mg^{2+} and K^{+} are the three most important ions that determine the survival and growth of *P. monodon* in inland saline water. The lack of a necessary mix of ions essential for osmoregulation (Casstille and Lawrence, 1981; Pequeux, 1995) such as K^{+} and Mg^{2+} has been shown to limit growth and survival of shrimp (Saoud *et al.*, 2003; Davis *et al.*, 2005). Collind and Russel (2003) achieved good survival and growth of *P. monodon* in inland saline water of 3 ppt salinity by increasing K^{+} content to 40 mg/l. This indicates that K^{+} plays an important role in the survival and growth of *P. monodon*. In order to grow shrimp in inland salinity waters, potassium salts are often added to fortify the potassium concentration (Liu, 2001; Allan and Fielder, 2002; Li *et al.*,2002). Similar results were obtained by Rahman *et al.* (2005) who determined that the survival of PL increased in saline water of 12.5 ppt when fortified with potassium chloride. Compared with the other essential ions, K^{+} is a minor constituent in brackish and freshwater (Horne, 1969) but it plays a major role in crustacean metabolism (Gross,1958; Bursey and Lane, 1971; Schmidt-Nielsen, 1990). When reared in K^{+}-deficient seawater, *L. vannamei* displayed anorexia, low activity, poor growth and even death (Zhu *et al.*, 2004). Water potassium concentration had a significant impact on the growth, molting, feeding and nutrient retention of the shrimp, while dietary potassium displayed little influence (McGraw and Scarpa, 2003). Dietary supplementation of potassium had limited effects on improving the growth of *L. vannamei* (Zhu et *al.*, 2006). If the water is low in Mg^{2+}, a number of agricultural products may be added to improve the ionic profile such as magnesium sulfate heptahydrate ($MgSO_4.7H_2O$: epsom salts) (Davis *et al.*, 2004.).

Based on the results of the present

study it could be concluded that in order to achieve good survival and growth of *P. monodon* in low-salinity culture, the salinity for PL stocking should be not less than 5 ppt and the ionic profiles should be similar to seawater. During the first 30 days, salinity should be maintained as long as possible or should not be allowed to drop too quickly. The minimum levels of Na^+, Mg^{2+} and K^+ during the culture period should be maintained not less than the concentration levels of seawater at 3 ppt.

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