

Project Number	VIE36 IF03		
Project Title	Moulting and growth of mud crab (<i>Scylla paramamosain</i>) larvae treated with extracts of neem tree (<i>Azadirachta indica</i>) and Cell Salts		
Vietnamese Institution	Research Institute for Aquaculture No. 2		
Australian Institution	Charles Darwin University		
Commencement Date	May 2006	Completion Date	December 2006
Objectives: To evaluate the effects of neem extracts and cell salts as an antibiotic replacement on mud crab (<i>S. paramamosain</i>) larvae in terms of moulting, growth, and survival rate from Zoea 1 to Megalopa. The effects of neem extracts and cell salts on the bacterial and fungal communities of the culture medium will also be monitored.			
Activities			
<ol style="list-style-type: none"> 1. Produce Zoea 1 of the mud crab <i>Scylla paramamosain</i> produced by spawning of the broodstock housed in the hatchery at Bac Lieu RSA. 2. Conduct Experiment 1 to determine the effects of neem extract on larvae (Z1)'s moulting and growth and water quality. 3. Conduct Experiment 2 to determine the effects of cell salts on larvae (Z1)'s moulting, growth and stamina. 			
Milestones			Expected Date
Nb r	Milestone Description	Deliverables	
1	CARD Contract Signed	<ul style="list-style-type: none"> • Research Agreement signed. Milestones and payment schedules in place 	April 2006
2	Research Report	<ul style="list-style-type: none"> • A summary report of all trial results, including the water quality and survival and growth rates of mud crab from Zoea 1 to Megalopa stage and analysis of economic and environmental benefits. 	December 2006

PROJECT COMPLETION REPORT

1. Introduction

In Mekong delta, mangrove forest distributes along the coastline of 700 km and it is a homeland for mud crab in tropical areas. The mud crab becomes potential to be alternative species from failed shrimp farming. Particularly, mud crab hatchery has been introduced and operated recently at Bac lieu, Tra vinh, Soc Trang, Ben Tre, Kien giang and Ca mau provinces. However, the survival from zoea stage to crablet stage 1 is low at approximately 5%. In addition, like shrimp rearing, the mud crab hatchery is using antibiotic as major treatment to prevent bacteria and fungi. However, antibiotic residues are harmful for consumers and are contributed to the development of bacterial resistant strains. For these reasons, the use of antibiotics in aquaculture is being discouraged. Replacement antibiotic

with anti-bacterial natural compounds is the best option to develop further aquaculture industry in Vietnam, particularly for new candidates such as mud crab.

Neem plus is a commercial medical product from Tattava's Herbs, USA. Each tablet of 500mg contains 450mg Neem tree extract. The Neem Plus was suggested to use for promoting healthy immune system for human. Tissue cell salts is a commercial product of Martin & Pleasance, VIC, Australia. Each tablet is 500 mg consisting of 0.5mcg of the Mineral Tissue Salt Calcium Phosphate in a lactose base. This product is used for the temporary relief of anaemia, bone diseases, and indigestion of human beings.

The Cell Salts and “neem products” have been successfully trialed with *Penaeus monodon* (Mustafa and Luong-van, 2004) for molting-safe and ecosecure treatment. However, these products have not been justified in rearing of mud crab larvae. In particular, a suitable dosage of these products was not tested. Therefore, this study reports on two experiment conducting in: 1) a preliminary trial to find out a suitable concentration of each product in rearing of mud crab larvae, and 2) replacement of antibiotics with Neem Plus, Tissue cell salt in mud crab larvae rearing.

Project objectives

The project aims to evaluate the effects of neem extracts and cell salts on mud crab (*S. paramamosain*) larvae in terms of moulting, growth, and survival rate from Zoea 1 to Megalopa. The effects of neem extracts and cell salts on the bacterial communities of the culture medium will also be monitored. The project objectives are achieved when there are 30-50% improvements of the treatments against the control.

2. Materials and Methods

2.1 General methods and materials

2.1.1 Site study

This trial was carried out at the Bac Lieu Experimental Station for Aquaculture belonging to the Research Institute For Aquaculture No.2.

2.1.2 Zoea preparation

Zoea 1 of species *Scylla paramamosain* was used in this study. The broodstocks were identified using Morphological Method described by Keenan *et al.* (1999) and were fed and managed from a protocol for mud crab hatchery prepared by the Bac Lieu Experimental Station for Aquaculture (BLESA). One best mud crab spawners was selected to produce Zoea 1 for testing.

2.1.3 Water preparation

Water was prepared following the basic steps for water preparation for mud crab hatchery. At the beginning, water was settled for 7 days, and then oxidized with 1ppm KMnO₄. When the water was settled and was clear, it was treated with chlorine (70%) of 30ppm for 3 days. After being chlorinated, water was neutralized with thiosulphate of 20 ppm, and then filled through a sand filter via UV light before being pumped into the rearing tanks.

2.1.4 Feeding

At the beginning, the larvae of mud crabs were fed with early Nauplius of Artemia (Vinh Chau, Vietnam) at rate of 8-10 Nauplii per zoea per feeding time. They were fed four times per day until Zoea 5. From Zoea 5, the home made feed was added at the rate of 10-15g/100,000 Zoea/time.

Table 1. Types of feed and feeding times at different larvae stages

Larvae stages	Type of feed	Feeding times
Zoea 1	Early Artemia	6h; 12h; 18h; 24h
Zoea 2 - Zoea 5	Artemia nauplii	6h; 12h; 18h; 24h
Zoea 5 – Crablets	Home made feed	6h; 12h; 18h; 24h

2.1.5 Maintain water quality

ET 600 (a component of vitamin and mineral, made by Long Sinh Company, Taiwan) at 1ppm was dissolved in the water of the rearing tank 30 minutes before zoea 1 was added. This product was used repeatedly at the end of each zoea stage. When larvae moulted to magalop stage, Shrimp loving at 2-5ppm (a premixtute of mineral, vitamin, cation and anion from Long Sinh Company, Taiwan) was used for reduction of toxic gas (eg. NH₃-N) in the water.

2.2. Materials and methods for specific experiments

2.2.1 Experiment 1

2.2.1.1 Time period

This experiment was carried out from 14th November to 10th December 2006. The experiment started with Zoea 1 and ended at Zoea 4 and megalop stages.

2.2.1.2 Rearing system

Seven tanks of 500L each were used in this experiment.

2.2.1.3 Experimental treatment

Neem plus and tissue salt were used at three levels (0.5 ppm, 1.0 ppm, and 2 ppm) (Table 2). The control treatment was a normal dosage of antibiotics as described in Table 3.

Table 2 The detail of three dosages of Neem Plus and Tissue Salt and antibiotics

Treatments	Neem Plus (ppm)	Tissue Salt (ppm)	Antibiotic
T1	0.5	-	-
T2	1	-	-
T3	2	-	-
T4	-	0.5	-
T5	-	1	-
T6	-	2	-
T7	-	-	As Table 2

Table 3 Description of the treatment using antibiotics (RAB7)

Stage	Actions
24 hours before rearing zoea 1	Use 2ppm A30 and 1ppm Shrimp Flavour to prevent protozoa and bacteria infection, respectively.
Zoea 2	Use 0.6 ppm Rifamicine and Mycostatine 1000UI per cubic meter to prevent bacteria and fungi.
Zoea 3	Use 2 tablets of Cotrimfoxazon 960 per m ³ to prevent bacteria in ingested system. After 6 hours, use 2ppm Biosubtyl-II to revert the normal ingestion system.
Zoea 5	Use 2ppm A30 and 1ppm Shrimp Flavour to prevent protozoa and bacteria infection, respectively. 3 days after, 0.6ppm Rifamicine and 1 tablet of Neo per 3 m ³ .

At the beginning, the Neem Plus and Tissue cell salts were added in the rearing tanks for 24 hours before mud crab larvae was supplied. The water exchange was applied at Zoea 3 stage with 50% of volum).

2.2.1.4 Exeprimental design and data analysis

This study was a preliminary evaluation of Neem Plus and Tissue Salt. Since budget limitation, all treatments were done in one replicate. All data was calculated using Excel (Micro office 2003).

2.2.2. Experiment 2: Replace anti-biotics using Neem Plus and Tissue cell Salts.

2.2.2.1 Time period: From 14th June to 5th July 2007.

2.2.2.2 Rearing system and density

15 fibreglass tanks of 300 L capacity each were used to rear mud crab larvae. Each tank was aerated with one air stone (Omega, Taiwan).

At the beginning, density was 60 Zoea 1 /L.

2.2.2.3 Experimental design

The experiment had 5 treatments; each treatment was done in triplicate. Description of treatments was presented in Table 4

Table 4 Description of treatments

Code	Description	Replicate
RAB1	Control treatment: did not use antibiotic, Neem plus or Tissue Salt.	3
RAB2	Normal antibiotic use for Zoea 1, Zoea 3 or Zoea 5 (kind of antibiotic and its dosage is presented in Table 5)	3
RAB3	Replace 50%? antibiotics with Cell salt. The antibiotic was used at Zoea 2 and Zoea 4 while Cell salt was treated at Zoea 1, Zoea 3 and Zoea 5.	3
RAB4	Zoea 1 was bathed with Neem Plus of 2 ppm for two hours. After that Neem plus of 0.5ppm was treated at the end of Zoea 1, Zoea 3, and Zoea 5	3
RAB5	Used Cell salt of 2ppm at the end of Zoea 1, Zoea 3, and Zoea 5	3

Table 5. Description of antibiotic treatment (RAB5)

Time	Description
24 hours before Zoea 1 were stocked in the rearing tanks	Shrimp Flavour of 1ppm was added to prevent fungus and bacteria.
Zoea 2	Rifacin 300mg: 2 tablets/m ³ ; Mycostatine 100000 UI: 1 tablet/2m ³ .
Zoea 3	Cotrimfoxazol 960mg 2tablets/m ³ was used to prevent ingestion disease. 6 hours after, Biosubtyl-II of 2g/m ³ was used to maintain enzyme in ingestion system
Zoea5	Shrimp Flavour of 1ppm was used to prevent fungus and bacteria. Three days after, Rifacin 300mg was added at rate 2 tablets/m ³ ; Mycostatine 100000 UI was used 1 tablets/2m ³ (or Neomycine 1 tablet/3m ³)

2.2.2.4 Water exchange

The water exchange was applied at the end of each zoea stage (zoea 2, 3, 4) with rate of 20% and 60% for Zoea 5.

2.2.2.5 Data collection

- Survival rate

Survival rate was recorded at the day of 7th, 14th, or 21th.

- RNA and DNA ratio

The estimations of RNA and DNA were done using the method of Aoki and Hase (1964). Contents of RNA and DNA of mud crab larvae body were determined at the beginning (Zoea 1) and the end (at 21 days of age).

- Water quality

- Temperature and pH were daily recorded at 8:00 and 15:00. Dissolved oxygen (DO) was daily recorded at 8:00.

- COD, NH₃, Nitrite, total bacteria, total vibrio were weekly recorded at day of age: 0, 7, 14, and 21

2.2.2.5 Statistical analysis

One way ANOVA was used to identify the significant difference at $\alpha < 0.05$. LSD was used to compare the difference between treatments.

3. Results and discussion

Experiment 1

Table 6. Number of zoea 1 at the beginning (No. at the beginning), zoea and megalop at the end (No. at the end), survival rate, percentage of megalop, and average body weight

Treatments	No. at the beginning	No. at the end	Survival rate (%)	Percent of Megalop	Average body weight (mg)
T1	40,000	29,094	72.7	0	2.7
T2	40,000	34,986	87.5	0	2.9
T3	40,000	27,606	69.0	0	2.3
T4	40,000	28,045	70.1	3.0	2.8
T5	40,000	36,340	90.9	2.5	2.5
T6	40,000	34,662	86.7	2.0	3.1
T7	40,000	21,735	54.3	3.0	4.8

The highest survival rate was obtained at T5 (90.9%) which was used Tissue cell Salts of 1 pm and the lowest value of 54.3% was observed at T7 which was used with antibiotics.

The Neem Plus treatment had survival rates (69-87.5 %) but larvae did not moult to Megalop stage. Probably, Neem Plus was against with the moulting activity of these mud crab larvae.

Nevertheless, the megalops were observed at all other treatments (T4, T 5, T6, and T7) ranging from 2.0-3.0 %. Therefore, Tissue cell Salts and antibiotics did impact similarly on moulting activities of mud crab larvae.

The smallest average body weight (2.3 mg) was also observed at treatment using the highest Neem Plus of 2 ppm while the highest value (4.8 mg) was detected when used antibiotics (T7).

The bigger larvae when used antibiotic can be a result of the lower survival rate. The highest dosage of Tissue cell Salts produced bigger larvae and higher survival rate.

Experiment 2

Table 7 shows the water parameters including: temperature, salinity, dissolved oxygen, and pH during the course of the experiment.

Table 7 Average values of temperature, salinity, dissolved oxygen, and pH during a course of experiment (mean \pm stdev*)

Parameter	Treatment				
	RAB1	RAB2	RAB3	RAB4	RAB5
Temperature at 8:00 (°C)	27.11 \pm 0.79	27.11 \pm 0.79	27.11 \pm 0.79	27.11 \pm 0.79	27.11 \pm 0.79
Temperature at 15:00 (°C)	27.99 \pm 1.04	27.99 \pm 1.04	27.99 \pm 1.04	27.99 \pm 1.04	27.99 \pm 1.04
Salinity (‰)	28.83 \pm 2.71	28.83 \pm 2.71	28.83 \pm 2.71	28.83 \pm 2.71	28.83 \pm 2.71
DO (mg/L)	7.24 \pm 0.72 ^a	7.18 \pm 0.74 ^a	7.22 \pm 0.73 ^a	7.23 \pm 0.75 ^a	7.22 \pm 0.70 ^a
pH	8.01 \pm 0.15 ^a	7.99 \pm 0.11 ^a	7.97 \pm 0.10 ^a	7.98 \pm 0.11 ^a	7.98 \pm 0.10 ^a

* Means within the same column having a similar superscript letter are not significantly different at 5% of significance (P>0.05)

In general, water temperature, salinity, DO, and pH values were not significantly different between treatments (P>0.05). Perhaps, the similar water parameters was due to the experiment was carried out indoor, with the same oxygen supply (1 air stone per tank), and the same water exchange. Although, all levels of DO, pH, and salinity were suitable for mud crab rearing, lower water temperature was observed at 18th day of experiment possibly because of rainy weather (Figure 1).

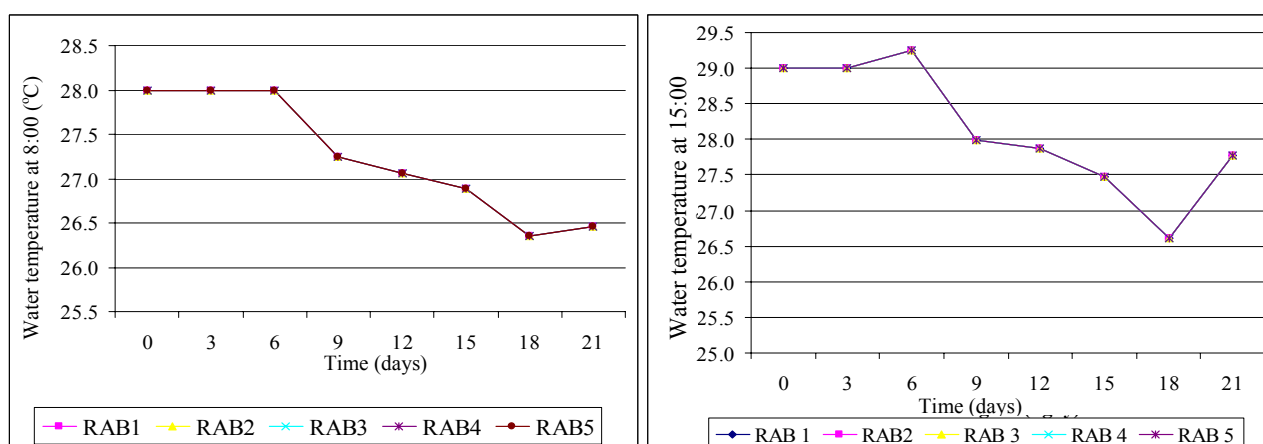


Figure 1 Water temperature at 8:00 and 15:00 varied during an experimented period

Table 8 Values of COD, NO₂-N, NH₃-N, total bacteria, yellow vibrio, and green vibrio (mean)

Source	COD	NO ₂ -N (mg/mL)	NH ₃ -N (mg/mL)	Total bacteria (CFU/mL)	Yellow vibrio (CFU/mL)	Green vibrio (CFU/mL)
Water supply	7.1300	0.0295	0.00026	300	11	0

Table 9 Values of COD, NO₂-N, NH₃-N, total bacteria, yellow vibrio, and green vibrio at 7days of age (mean±stdev*, n=3)

Source	COD (mg/mL)	NO ₂ -N (mg/mL)	NH ₃ -N (mg/mL)	Total bacteria (CFU/mL)	Yellow vibrio (CFU/mL)	Green vibrio (CFU/mL)
RAB1	14.193±2.482 ^{ab}	0.002±0.004 ^a	0.188±0.068 ^{ab}	1533.33±952.48 ^b	162.33±95.00 ^{bc}	27.00±1.43 ^{bc}
RAB2	13.553±6.952 ^{ab}	0.003±0.001 ^a	0.238±0.040 ^{ab}	2866.67±2227.85 ^a	211.00±57.03 ^{ab}	49.00±11.88 ^{ab}
RAB3	19.533±4.680 ^a	0.000±0.001 ^a	0.309±0.147 ^a	1433.33±802.08 ^b	246.33±84.10 ^{ab}	67.00±12.08 ^a
RAB4	12.847±1.378 ^{ab}	0.002±0.003 ^a	0.283±0.061 ^a	1533.33±838.65 ^b	325.33±49.36 ^a	33.33±12.53 ^b
RAB5	7.887±3.697 ^{bc}	0.003±0.003 ^a	0.102±0.033 ^{bc}	2366.67±1598.57 ^{ab}	221.67±46.69 ^{ab}	32.33±11.73 ^b

* Means within the same column having a similar superscript letter are not significantly different at 5% of significance (P>0.05)

Table 10 Values of COD, NO₂-N, NH₃-N, total bacteria, yellow vibrio, and green vibrio at 14days of age (mean±stdev*, n=3)

Source	COD (mg/mL)	NO ₂ -N (mg/mL)	NH ₃ -N (mg/mL)	Total bacteria (CFU/mL)	Yellow vibrio (CFU/mL)	Green vibrio (CFU/mL)
RAB1	16.433±3.070 ^a	0.006±0.002 ^a	0.042±0.026 ^{bc}	8733.33±677.37 ^a	378.89±123.07 ^b	361.78±95.10 ^a
RAB2	11.263±4.109 ^{ab}	0.005±0.002 ^{ab}	0.086±0.039 ^{ab}	12833.33±2218.86 ^a	465.00±161.37 ^b	74.33±24.58 ^c
RAB3	16.130±4.364 ^{ab}	0.007±0.002 ^a	0.051±0.041 ^b	11955.56±5787.85 ^a	434.11±76.40 ^b	112.00±13.38 ^b
RAB4	12.493±5.058 ^{ab}	0.005±0.001 ^{ab}	0.11±0.010 ^{ab}	10266.67±5315.39 ^a	578.33±185.00 ^a	177.33±20.39 ^b
RAB5	8.097±5.302 ^{bc}	0.002±0.002 ^{bc}	0.06±0.001 ^b	11400.00±3347.14 ^a	406.22±148.67 ^b	157.00±30.51 ^b

* Means within the same column having a similar superscript letter are not significantly different at 5% of significance (P>0.05)

Table 11 Values of COD, NO₂-N, NH₃-N, total bacteria, yellow vibrio, and green vibrio at 21 days of age (mean±stdev*, n=3)

Source	COD (mg/mL)	NO ₂ -N (mg/mL)	NH ₃ -N (mg/mL)	Total bacteria (CFU/mL)	Yellow vibrio (CFU/mL)	Green vibrio (CFU/mL)
RAB1	14.797±0.849 ^a	0.066±0.051 ^a	0.14±0.12 ^b	10166.67±480.35 ^a	577.67±115.99 ^a	216.67±96.67 ^a
RAB2	10.480±2.833 ^a	0.060±0.024 ^a	0.17±0.12 ^b	9166.67±351.19 ^{ab}	562.33±97.83 ^a	15.33±17.90 ^c
RAB3	12.767±6.585 ^a	0.071±0.008 ^a	0.32±0.02 ^a	9111.00±2691.55 ^a	299.33±87.12 ^b	82.67±47.08 ^b
RAB4	13.313±2.358 ^a	0.042±0.031 ^a	0.14±0.02 ^b	8200.00±1058.30 ^b	675.67±99.71 ^a	158.00±68.48 ^a
RAB5	13.150±2.338 ^a	0.081±0.062 ^a	0.31±0.03 ^a	9366.67±976.64 ^a	525.33±64.72 ^a	112.00±13.11 ^b

* Means within the same column having a similar superscript letter are not significantly different at 5% of significance (P>0.05)

Tables 8, 9, 10, and 11 shows that values of COD, nitrite, amonia, total bacteria, and yellow vibrio were lower than tolerant levels for crustacean larvae rearing (Boyd & Tucker 1998).

Seven days after stocking larvae, the highest COD value was 19mg/L in RAB3 (combination between Tissue salt and antibiotic). Values for nitrite were similar for all treatments. The highest values of NH₃-N were observed at treatments RAB3 and RAB 4 (P>0.05). Total bacteria increased dramatically at all treatments, in particular green vibrio (a cause of disease) developed in all treatments (Table 9).

Table 10 shows that 14 days after being stocked, contents of COD, NO₂-N, NH₃-N increased highly but their values were within the range of tolerant (Boyd & Tucker 1998). The highest COD value of 16.433 mg/L was detected at the control treatment (RAB1). In general, high amount of total bacteria was presented in all treatments. The highest value of green vibrio was observed at RAB1 while it was lowest in the antibiotics treatment RAB2. The medium green vibrio levels were found in all other treatments including those of Neem Plus, Tissue Salt, or combination between Tissue cell salts and antibiotics.

A similar result was found at 21 days of growth (Table 11). In summary, during the couse of the experiment, values of water quality parameters (water temperature, pH, DO, COD, nitrite, and amonia) were lower than tolerant levels for mud crab larvae. However, total bacteria density andgreen vibrio were detected in all treatments from 7 days of age onwards. This could be a cause for the decline of larvae number and survival rates (Tables 12 and 13).

Decline of number and survival rate of mud crab larvae

Table 12 A number of larvae at different stages (mean ± stdev*, n=3)

Treatment	Larvae stages				
	Zoea 2	Zoea 3	Zoea 4	Zoea 5	Megalopa
RAB1	13033.3±665.8 ^a	10866.7±1101.5 ^a	7800.0±2291.3 ^b	1733.3±450.9 ^c	149.0±56.9 ^c
RAB2	13966.7±351.2 ^a	12300.0±1014.9 ^a	10700.0±1179.0 ^a	8666.7±896. ^{3a}	869.7±99.5 ^a
RAB3	13266.7±862.2 ^a	11600.0±1058.3 ^a	10133.3±757.2 ^{ab}	8333.3±680.7 ^a	815.0±146.2 ^a
RAB4	11766.7±1011.6 ^b	8366.7±1069. ^{3b}	6600.0±655.7 ^b	4600.0±721.1 ^b	144.3±80.6 ^c
RAB5	10666.7±208.2 ^b	7600.0±556.8 ^b	5866.7±832.7 ^b	3800.0±608.3 ^b	228.3±118.4 ^b

* Means within the same column having a similar superscript letter are not significantly different at 5% of significance (P>0.05)

Table 13 Survival rate of larvae at different stages (mean± stdev*, n=3)

Treatment	Larvae stages				
	Zoea 2	Zoea 3	Zoea 4	Zoea 5	Megalopa
RAB1	72.4±3.7 ^{ab}	60.4±6.2 ^a	43.3±12.7 ^{bc}	9.6±2.5 ^c	0.8±0.3 ^b
RAB2	77.6±1.9 ^a	68.3±5.6 ^a	59.5±6.6 ^a	48.2±5.0 ^a	4.9±0.6 ^a
RAB3	73.7±4.8 ^a	64.5±5.9 ^a	56.3±4.2 ^{ab}	46.3±3.8 ^a	4.5±0.8 ^a
RAB4	65.4±5.6 ^{bc}	46.5±6.0 ^b	36.7±3.6 ^c	25.6±4.0 ^b	0.8±0.5 ^b
RAB5	59.3±1.2 ^c	42.2±3.1 ^b	32.6±4.6 ^c	21.1±3.4 ^b	1.3±0.7 ^b

* Means within the same column having a similar superscript letter are not significantly different at 5% of significance (P>0.05)

Tables 12 and 13 show that number of larvae and survival rate decreased significantly from the beginning to the end of the experiment. Particularly, the survival rate declined suddenly from Zoea 5 to Megalop. Probably, low temperature at days 16, 17, and 18 (Figure 1) affected on the low survival rate of larvae.

At megalop stage, the highest survival rates were observed at treatments using antibiotics (RAB2) and combination between Tissue salt and antibiotics (RAB3).

Ratio between ribonucleic acid (RNA) and deoxyribonucleic acid (DNA)

Table 14 RNA/DNA ratios (values as means ± SD, N=3)

Treatments	RNA/DNA ratio*
RAB1	4.9±2.3 ^{cd}
RAB2	0.5±0.2 ^{ab}
RAB3	2.0±2.4 ^{bc}
RAB4	4.9±1.5 ^{cd}
RAB5	8.1±3.9 ^d
Initial larvae (Zoea 1)	6.3± 2.4

* Means within the same column having a similar superscript letter are not significantly different at 5% of significance (P>0.05)

The RNA/DNA ratio (given in Table 14) showed a decline in value in all treatments except RAB5 and did not substantiate the survival data (Table 13). The highest survival rates to Zoea 5 and Megalopa were at RAB 2 and RAB 3 (Table 13). However, the RAB2 and RAB 3 treatments show the lowest RNA/DNA ratios (Table 14). It is noteworthy that the presence of antibiotics severely depressed the RNA/DNA ratio (RAB

2 and RAB3). The addition of the neem product (RAB4) did not seem to affect the ratio since the ratio was not significantly different from the control (RAB1) treatment. The addition of tissue cell salts (RAB5) markedly increased the RNA/DNA ratio, which is similar to the work of Mustafa and Luong-Van (2004) for *Penaeus monodon*.

Although the RNA/DNA ratios were used for the assessment of the nutritional condition of hatchery-reared *Penaeus monodon* post larvae (Mustafa and Luong-Van 2004) and *Macrobrachium rosenbergii* juveniles (Behanan and Mathew 2004), they were not suitable for use in the present work. It appears that substantial refinements have to be made to the method before it can be used.

4. Conclusions

- At the end of the experiment 2, there were no differences between treatments: control treatment (did not used antibiotics, Neem plus, or tissue cell salts), Neem plus, tissue cell salts, antibiotics, or the combination between antibiotics and tissue cell salts in terms of COD, NO₂-N, NH₃-N, total bacteria, and yellow vibrio density.
- Using tissue cell salts (RAB 5) reduced green vibrio density better than Neem plus (RAB 4) and controlled treatments (RAB 1) but it was not better than that of using antibiotics alone or antibiotics and tissue cell salts combination.
- Using single Neem plus or tissue cell salts did not improve survival rate of megalop than that of antibiotics or antibiotics and tissue cell salts combination.
- Using Neem plus and tissue cell salts yielded higher ratios between RNA and DNA than that of using antibiotics or antibiotics and tissue cell salts combination.
- In sum, although the results of this investigation did not satisfy the bench-mark set in its objectives, they showed that neem and tissue cell salts products have potentials in replacing the antibiotics currently being used in mud crab hatchery. In particular, a 50% replacement of antibiotics with tissue cell salts (see RAB3) yielded similar results as those of 100% antibiotics (see RAB2). However, in order to realize these potentials substantial further work has to be done.

5. References

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