THE PROJECT STUDY ON VALUE-ADD FRESH WATER AQUACULTURE BY THE THIRD WATER IN THE KINGDOM OF CAMBODIA

FINAL REPORT

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JAPAN INTERNATIONAL COOPERATION AGENCY (JICA)

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INTEM CONSULTING, INC.

Preface

In order to confirm the applicability of the third water which has been developed by the Okayama University of Science, Japan International Cooperation Agency (JICA) carried out basic information collection and verification survey in March 2013 targeting three countries of Thailand, Cambodia, and Laos where JICA has been implementing the aquaculture project.

As a result, in order to examine the possibility of high-value-added of fresh water aquaculture utilizing the third water, the experiment of seed production during zoea to post larvae of giant fresh water prawn is migration between fresh water and blackish water implemented under the support of the Okayama University of Science, in Marine Aquaculture Research and Development Center (MARDeC) in Cambodia in 2013. In particular, under same condition of facility and breeding method, within zoea to post larvae (PL) of giant fresh water prawn is migration between fresh water and blackish water, the effectiveness of the third water in seed production was examined by some types of the third water. As a result, the third water Type C showed as higher and/or equivalent PL survival rate as control. It was confirmed the high efficacy as breeding water for seed production of giant fresh water.

Based on the results of the last year, JICA implemented the seed prodution experiment at farmer level in Cambodia to finalize as technique and had accomplished this report after analysis.

We hope that this report will be utilized to parties involved in international cooperation in the fisheries and aquaculture sector.

At the end, we sincerely would like to give our gratitude to whom it may concern had cooperated and supported

January 2015

Japan International Cooperation Agency
Rural Development Department
Director General
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Abstract

1. Purpose

On the basis of result in last year, this project study was planned to verify practical utility of the recirculating filter system using the third water type C in giant freshwater prawn seed production in inland area of Cambodia (around Takeo Province). The experiment aims to finalize the technique to be available on farm level as well as to verify the possibility of extension of seed production.

2. Schedule

This project study originally had been planned from July to November 2014. However, due to delay of arrival of some equipment from Japan, the experimental seed production trial was implemented from August to December 2014, 1 month behind schedule.

3. Experiment group

This project study has introduced the sun dry salt to reduce the third water cost and the solar panel system to adapt the status of the power supply in Cambodia. Because these had been first trial in Cambodia, in order to diminish risk, the experiment group was planned as the following.

Table Target farmer and experiment plot

	Target farmer	Village	Commune	District	Experiment plot	Electricity	Remark
A	Prum Vat[※1]	Prey Sambo	Ankorborey	Ankorborey	The Third water type C prepared in Japan	Public electricity + generator	Confirmation the seed produce at farmer level
B	Hang Heang	Chork	Rokakhnong	Donkeo	The Third water type C prepared by local sun-dried salt	Public electricity + generator	Confirmation the seed produce by local sun-dried salt
C	Van Po	Ou Phot	Angtasom	Tramkak	The Third water type C prepared in Japan	Solar system + generator	Confirmation the seed produce with solar system

4. Result

The average of the survival rate in farmer C (Mr. Van Po) site was 46.56%, it was exceeding 39.28% which was the results of experiment carried out last year in 220L tank using the third water type C. On the other hand, post larvae (PL) was not harvested in farmer A (Mr. Prum Vat) site and farmer B (Mr. Hang Heang) site because of mass mortality before metamorphosis in PL. The survival rates of zoea stage were around 1% respectively.

Table Seed production survival rate in each site

		Start (head)	Harvest (head)	Survival Rate(%)
Farmer A Prum Vat	TankNo,1	50,000	1,598	3.20
	TankNo,2	50,000	846	1.69
	TankNo,3	60,000	696	1.16
	Total	160,000	3,140	1.96
Farmer B Hang Heang	TankNo,1	60,000	756	1.26
	TankNo,2	60,000	935	1.56
	TankNo,3	60,000	456	0.76
	Total	180,000	2,147	1.19
Farmer C Van Po	TankNo,1	60,000	33,084	55.14
	TankNo,2	60,000	29,310	48.85
	TankNo,3	60,000	21,411	35.69
	Total	180,000	83,805	46.56

5. Consideration

Among three experimental farmers only farmer C (Mr. Van Po) harvested PL successfully. The result showed a possibility of seed production of giant fresh water prawn under well-controlled condition although other two sites failed harvesting PL. It is considered that the water recycling filter system with the third water type C and solar power has potential of seed production of giant fresh water prawn even in undeveloped inland area without seawater or public electricity.

List of abbreviations

AIM	Aquaculture of Indigenous Mekong Fish Species
AIT	Asian Institute of Technology
EMS	Early Mortality Syndrome
FAIEX	Freshwater Aquaculture Improvement and Extension Project in Cambodia
GM	Greater Mekong
JICA	Japan International Cooperation Agency
MARDeC	Marine Aquaculture Research and Development Center
PL	Post larvae
ppm	parts per million
psu	practical salinity unit

Activity photo-1

Giant fresh water prawn
(*Macrobrachium rosenbergii*)

Zoea of giant fresh water prawn
(stage IV)

Post larvae (PL)of giant fresh water
prawn

Description of the experimental
design for the candidate farmer
Farmer B (Mr. Hang Heang)

Measurement for hatchery
Farmer A (Mr. Prum Vat)

Constriction of hatchery
Farmer A (Mr. Prum Vat)

Hatchery
Farmer C (Mr. Van Po)

Installation of tanks
Farmer B (Mr. Hang Heang)

Filter (coral sand)
Farmer B (Mr. Hang Heang)

Plumbing the filter tank
Farmer C (Mr. Van Po)

Installation of solar panel
Farmer C (Mr. Van Po)

Confirmation of electricity by solar
panel
Farmer C (Mr. Van Po)

Activity photo-2

Sediment occurred were mixed the third water material to groundwater

Transportation of city water Farmer A (Mr. Prum Vat)

Generators (24 hours a day)
Farmer A (Mr. Prum Vat)

Concentrated nitrifying bacteria
Farmer A (Mr. Prum Vat)

Introdution of Ammonia and nitrite measurement
Farmer B (Mr. Hang Heang)

Ammonium chloride addition to the filter tank
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Giant fresh water prawn markets in Takeo city

Broodstock selection

Broodstock with eyed egg

Broodstock with non-eyed egg

Broodstock breeding
Farmer C (Mr. Van Po)

Counting of hatching zoea
Farmer B (Mr. Hang Heang)

Activity photo-3

Warmth of broodstock tank
Farmer A (Mr. Prum Vat)

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Farmer A (Mr. Prum Vat)

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Farmer B (Mr. Hang Heang)

Confirmatin of zoea stage
Farmer C (Mr. Van Po)

Bacillariophyta in breeding tank
Farmer B (Mr. Hang Heang)

Input of shelter
farmer C (Mr. Van Po)

Lecture on intermediate breeding

Harvest by scope net
Farmer C (Mr. Van Po)

PL weight measurement
Farmer C (Mr. Van Po)

Release of PL to the intermediate breeding pond
Farmer C (Mr. Van Po)

Confirmation of zoea after mass mortlity
Farmer A (Mr. Prum Vat)

Lecture of seedling production and aquaculture situation of Giant fresh water prawn in Thailand

1 Background and purpose of the project study

1.1 Background of the project study

In recent years, aquaculture system with the third water developed by Okayama University of Science has been gathering attention. The third water can be prepared by adding minimum electrolyte in freshwater. Based on the research implemented by Okayama University of Science, the third water improves growth of fish in aquaculture moreover it is also available for several species of marine fish. Fishes grown up by third water in Okayama University of Science nowadays were acceptable to market and obtained certain reputation.

At the beginning in order to verify the applicability of the third water, Japan International Cooperation Agency (JICA) dispatched survey mission for the basic information collection March 2013 in three target countries such Thailand, Cambodia, and Laos where JICA has been implementing the aquaculture project. As a result, JICA mission team suggested that the third water has a possibility of advantage in aquaculture as follows.

(1) The third water has potentials to contribute significantly to improve growth and survival rate which are the most important for aquaculture fish and crustacean and also disease prevention.
(2) Production cost is able to be reduced in nursing larvae of Giant fresh water prawn (*Macrobachium rosenbergii)* in inland area because it doesn't need to use salt water transported from a long distance.
(3) The third water could promote growth rate of juvenile stage for Marble Goby, which generally show slow growth rate.
(4) The third water could be effective against disease such EMS that causes serious problem in farming of black tiger shrimp (*P. monodon*) and white shrimp (*P. vannamei*) species.

In light of this situation, JICA made a plan to conduct "Project Study on Value-add Freshwater Aquaculture" in Cambodia to verify effectiveness of the third water targeting on Giant fresh water prawn (*Macrobachium rosenbergii)* in 2013.

Within zoea to post larvae (PL) of giant fresh water prawn is migration between fresh water and blackish water, the effectiveness of the third water in seed production was examined. The experiment was implemented with 9 types of the third water throughout 2 times experiment in Marine Aquaculture Research and Development Center (MARDeC) constructed by Japan's grant aid. As a result, the third water type C showed as higher and/or equivalent PL survival rate as control. It was confirmed the high efficacy as breeding water for seed production of giant fresh water and has been suggested the possibility of seed production of giant fresh water prawn that does not depend on the area.

1.2 Purpose of the project study

In Southeast Asia and Africa area, JICA has been conducting a technical cooperation relating to freshwater and sea water aquaculture, but fish production is greatly affected by natural conditions such as flooding, drought and other unpredicted weather exchange that are currently caused by global warming.

Responding to these issues, in order to beforehand with the food crisis due to environmental exchange and population growth humanity and to encourage a sustainable aquaculture production in the above areas, JICA aimed at the development and the extension the aquaculture technology of less susceptible to natural conditions and location. Under such situation, with the technical assistance related to the recirculating filter system using the third water from Okayama University of Science, JICA has decided to implement the project study on value-add fresh water aquaculture by the third water in the kingdom of Cambodia in 2013 and 2014.

On the basis of result in last year, this project study established the recirculating filter system using the third water type C in giant freshwater prawn seed production in inland area of Cambodia (around Takeo province) to verify its effectiveness on farm. Throughout the seed production trails, the experiment aimed to prepare basic protocol of giant freshwater prawn seed production to be utilized for further extension of seed production in inland area.

2 The third water and water recycling filter system

2.1 The third water

It is said that the body salt concentration substantially equal in marine and freshwater fish, but the method of osmoregulation in freshwater and marine fish are different. The body of marine fish adjusts the salt concentration in the cell. Because the body fluid is 1/3 of the salinity of seawater, the dehydrating action functions. Therefore, marine fish drinks a large amount of sea water and actively excrete excess salts. Small monovalent ions, such as sodium and chlorine are excreted by gills. Larger multivalent ions, especially calcium and magnesium which are abundant in seawater, are excreted by the urine. Freshwater fish has the opposite function, freshwater fish is keeping the salt concentration in the cell by uptake large quantities monovalent ions from gills and excrete large amount of urine with low concentration. Energy of this osmoregulation accounts for approximately 30% of the total energy metabolism.

Seawater contains about 60 types of ions such as sodium chlorine ions and carbonate etc. The third water developed by Okayama University of Science is functional water to adjust the ions balance involved in the metabolism of the fish such as sodium, potassium, and calcium. And it has been identified and adjusted the minimum ions for breeding fish and is able to suppress the energy consumption in the osmotic pressure of the fish due to adjust the same concentration of fluid of the fish body for marine and freshwater fish. That is the energy from feed and can be used for growth. Other thing, pathogens in the seawater and freshwater have the osmotic pressure corresponding to survive in each environment. But, in the third water, it is supposed that pathogens is harmful to fish may be non-active because specific gravity is different in seawater and freshwater.

2.2 Water recirculating filter system

In aquaculture using sea water, there are two methods such as the sea culture and the inland culture roughly. In the sea culture, there are a limitation of suitable area, an influence of season, weather and red tide, and a management issue such as net cleaning and replace of dead fish.

On the other hand, inland culture is a technique that is beginning to be introduced in some private farm and seed production centers in recent years. In inland culture, there are two methods such as the water flowing system and the water recycling filter system. Main aspect in the water flowing system and the water recycling system aquaculture are shown in Table 2.1.

Inland culture system has remained issues of the cost aspect such as initial cost and electricity cost. But the water recycling filter system aquaculture specially be attracted attention as the possibility of anthropogenic management, no risk of the entry of the disease organism, few limitation of place and few influence to exterior environment.

Table 2.1 Main aspect in the water flowing system and the water recycling system aquaculture

Article	Water flowing system	Water recycling system
Facility	Water pumps, water tank etc.	Circulation pump, water tank, filtration tank, etc.
Locational conditions	Coastal area	Unlimited (can be inland)
Environmental load	Environmental load by residual feed	No environmental load
Disease Control and Prevention	Pathogen invasion from the sea	No risk
Temperature control	Adjustable in a measure	Adjustable
Growth rate	Slow except in proper water temperature	Fast (possible temperature control)
Production cost	Cheapness	High (running cost)

Japanese Fisheries Agency (2013)

2.3 Research performance by Okayama University of science

Okayama University of Science has conducted experiments with 10 species including flounder, grouper, tiger puffer and striped jack so far, consequently it was verified that third water can be applicable to fish culture by combination with water recirculating system under no medication.

Trials targeted the tiger puffer operated in 2012-2013 were shipped 2,181 fish after 18 months using 20kL tank for 2 groups. This operation was carried out without medicine and water exchange (evaporation is excluded).

Table 2.2 Result of tiger puffer production in water recycling system using the third water

Initial number of fish	Shipping volume	Survival rate (%)
3,426	2,181	63.66
Initial fish weight (kg)	Shipping fish weight (kg)	Weight gain (kg)
11.484	1,890.409	1,878.925
Feed amount (kg)	Feed efficiency (%)	Conversion coefficient
2,986.668	62.91	1.59

Okayama University of Science (2013)

It has definite advantages such as "Occurrence of fish disease is suppressed", "Breeding water is cheap", "growth rate is improved", “Fish culture activity does not depend on the weather condition", “Environmental load is reduced” and "Breeding can be done anywhere". Okayama University of Science is currently also researching is underway to target the improvement of productivity and new fish species.

3 Biology and situation of aquaculture for giant fresh water prawn

3.1 Biology of giant fresh water prawn

Giant fresh water prawn is habiting in tropical and subtropical regions of Southeast Asia. It usually inhabits freshwater but also it rarely is observed in brackish water. It inhabits river, especially near to coastal zone (it is observed even in inland away 200 km from the coast), lake, canal and rice field (Claudio Chávez Justo, 1990[1]).

Seeing life-cycle, giant fresh water prawn is mostly living in freshwater except for the larval stage that lives in brackish water. Typically, the maturity, mating and spawning are performed in freshwater. The fertilized egg is not released into water, is adhered with uropod (swimming appendages) unlike the case of kuruma prawn (*Marsupenaeus japonicas*).

It needs about 18 days for hatching and it is mainly influenced by water temperature. In this ovulation period, female starts to immigrate toward downstream of the river, the egg hatches in estuary area. Larval stage starts from the stage of Zoea. Newly hatched larvae require necessarily brackish water. Larva becomes a post larva (PL) after 11 distinct larval stages. PL begins moving to upstream the river and grows to adult.

3.2 Utilization of giant fresh water prawn

Giant freshwater prawn is an important fishery resource in Southeast Asia and other countries. It is sold not only for home consumption but also for food service industry. The major production countries in South-east Asia such Vietnam, Thailand and Bangladesh etc. export it to other country since it is highly commercial value. It is sold mainly for local consumption in Cambodia but also the frozen or the alive prawn are transacted by retail dealer to urban areas such Phnom Penh and Siem Reap. Alive giant fresh water prawn is often seen in water tank of restaurant to be served with fresh. It is not eaten in raw fresh due to parasite. Most people prefer cooked prawn such as boil, roast, and fry. It is common especially in local and Chinese restaurant. Giant fresh water prawn is also sold in aquarium shop as an ornamental fish beyond that.

Market price of giant fresh water prawn in Takeo province is shown Table 3.1. It is divided into three grades that grade I is average weight 220g grade II is about 120g, grade III is about 40g. Although grade I is generally 28 USD / kg, it is increased 32-35 USD / kg in Khmer New Year and water festival. Other fish, for example Regular price of grade I is 14 times higher than silver barb and common carp (2-2.5 USD/kg).

[1] Claudio Chávez Justo, 1990. The aquaculture of shrimp, prawn and crayfish in the world : basic and technologies.. Midori Shobo.

Table 3.1 Price of Giant fresh water prawn in Takeo province

Grade	Average Wight	Usual price	Special price
I	220g	28USD/kg	32-35USD/kg
II	120g	22USD/kg	—
III	40g	17USD/kg	—

Hearing investigation (2014)

3.3 Situation of aquaculture for giant fresh water prawn in Cambodia

Seed production of giant fresh water prawn was succeeded for the first time by Shao-Wen Ling and other researchers in Penang, Malaysia in 1961. After the series of research, aquaculture of this species in industry level attracted people’s attention. Subsequently basic technology to produce this species has been expanded and developed in Southeast Asian countries such as Vietnam and Thailand and Hawaii, China and Japan.

History of seed production of giant fresh water prawn in Cambodia has started by Dr. Shiro HARA, who is in charge of team leader for consultant in this project, during the Freshwater Aquaculture Improvement and Extension Project in Cambodia Phase1 in 2006. He succeeded the mass seed production for the first time in Cambodia at national aquaculture center in Prey Veng province by using technology referred to method of Can Tho University. Afterward seed production has been carried out in private farm in Takeo province. This species is drawing people’s attention as aquaculture target species in recent years.

But the amount of aquaculture production of giant fresh water prawn is still lower than other fishes in Cambodia (Table 3.2). As this factor, (1) "it is not profitable because commercial feed has to be used from post larvae to market size", and (2) "the difficulty of management in seed production". In factor (2), it has been indicated as specific problem due to water transportation cost to the inland, the occurrence of disease, the survival rate of larvae is unstable. It is expected to improve to (2) by using the third water.

Table 3.2 Aquaculture production volume for fresh water fish and giant fresh water prawn in Cambodia

(unit：ton)

	2008	2009	2010	2011	2012
Freshwater prawn	30	110	120	140	140
Common carp	100	120	140	170	190
Silver barb	900	1,230	1,400	1,650	1,700
Tilapia	7,000	8,750	10,500	12,600	13,000
Silver carp	1,150	1,450	1,700	2,000	2,100

FISHSTATJ (2014)

4 Summary of this project study and result in 2013

4.1 Summary of this project study

In this project study, the following overall goal project goal, output, and activity has been set.

(1) Overall goal

Seed production of giant fresh water prawn using the third water is carried out by seed production farmer in inland of Cambodia.

(2) Project goal

Water recycling filter system using third water for seed production of giant fresh water prawn is established.

(3) Output

In order to achieve the above goal, outputs are as follows.

Output1: Larvae of giant fresh water prawn are produced by the third water.

Output2: Proper the third water for seed production of giant fresh water prawn is selected

Output3 Water recycling filter system using third water for seed production of giant fresh water prawn is established with local resources.

(4) Activity

The project research in 2013 had been carried out activities to aim at the achievement of outcomes 1, 2. This year, activities towards the achievement of output 3 had been carried out.

Output1: Larvae of giant fresh water prawn are produced by the third water.

1) Experiment tanks and equipment is set up and adjusted.
2) Seed production using the third water is carried out.
3) Broodstock is managed and hatched out.
4) Larvae are cultured by the third water until post larvae

Output2: Proper the third water for seed production of giant fresh water prawn is selected

1) Under same condition of facility and breeding method, the performance such as survival rate, total length and cost etc. are examined in each type of the third water.
2) Base on the seed production of giant fresh water prawn using the third water, it is compared with standard method.
3) Cost of the seed production of giant fresh water prawn using the third water is calculated, and is compared with standard method.

Output3: Water recycling filter system using third water for seed production of giant fresh water prawn is established with local resources.

1) Seed production system with saving energy and efficient is established.
2) Experiment tanks and equipments is set up and operated in target farmers. One part of farmer site is set up solar panel system and operated.
3) The third water using sun dry salt is produced.
4) Broodstock is managed and hatched out.
5) Farmer mainly culture until develop to post larvae.
6) Cost of the seed production of giant fresh water prawn using the third water is calculated, and is compared with standard method.
7) In consideration of the above, extension method is planned.

4.2 Experiment contents in 2013 and 2014

The objective of the project in 2013 was to find the suitable third water type for culturing and inspect the effectiveness of seed production by using the third water for giant fresh water prawn from zoea to post larvae. The objective of the project in 2014 is to inspect the seed production of giant fresh water prawn with the third water at the farmer level. Table 4.1 shows the comparison of the experimental study in 2013 and 2014.

Table 4.1 Contents of Experimental study in 2013 and 2014

	2013	2014
Main Objective	To select the third water for cultivating Giant fresh water prawn and verify larval rearing	To verify the seed production of Giant fresh water prawn with the Third water at the farmer level
Experimental site	Marine cultured development center in Sihanouk Ville(MARDeC)	Fish farmer's facilities in Takeo Province
Role-sharing	(1)Conductor: Consultant (2)Advisor: Okayama University of Science (3)Counterpart (C/P): The MARDeC staff	(1)Conductor: Seed farmers and Consultant (2)Advisor: Okayama University of Science (3)Counterpart (C/P): Extension officer, The MARDeC staff
Kinds of the breeding water	Diluted seawater and 9 types of the third water	The third water(Type C) prepared in Japan and prepared by local sun-dried salt
Capacity of tank (Scale of breeding)	220L/tank	1kL/tank×3 tanks
Facility for breeding	Indoor breeding facilities in MARDeC where seawater, fresh water and electricity are available	The farmer house site (electricity and water is depend on farmer). In 1 of 3 sites solar system shall be installed.

4.3 Experiment result in 2013

The project was conducted from 8 August 2013 to 29 October 2013 in the Marine Aquaculture Research and Development Center (MARDeC). The design of experiments is shown in table 4.2. Zoe of giant fresh water prawn were cultured in tank with water recirculating system until post larvae (PL) and the survival rate in each group was compared to analyze after harvesting PL.

Table 4.2 Experiment group in 2013

	1st experiment	2nd experiment
Control	dilution sea water	dilution sea water
The third water	Standard	Type A
	Type A	Type A-H*
	Type B	Type C
	Type C	Type C-H*
	Type D	Type E
		Type E-H*

*H is high concentration

According to the results of 2 times of experiment, PL survival rate in control and the third water type C were 31.6% (first experiment) and 36.4% (first experiment) respectively. The third water type C showed higher or equivalence survival rate than control. The whole survival rate in this experiment was not so high compared to survival rate as usual seed farmer because the larval rearing was conducted in small-scale using 220L tank to compare variety type of third water. This experiment has suggested that the third water type C could be applicable to seed production of giant fresh water prawn.

In this experiment, it was confirmed that water quality especially ammonia and nitrite were unsteadiness. In case of seed production by water recycling filter system, it was suggested that 1) to put fresh nitrifying bacterium into the filter system, 2) to use adsorbent material to reduce the ammonia, and 3) to select the filter tank to adapt rearing tank scale.

5 Schedule of the project study

5.1 Experiment schedule

This project study was planned from July to November 2014. Because of delay about 1 month the some equipment arrival from Japan, this project implemented from August to December 2014 (Table 5.1). Based on the lessons of the last year, preparation period of Bio filter system was thoroughly taken as particular attention.

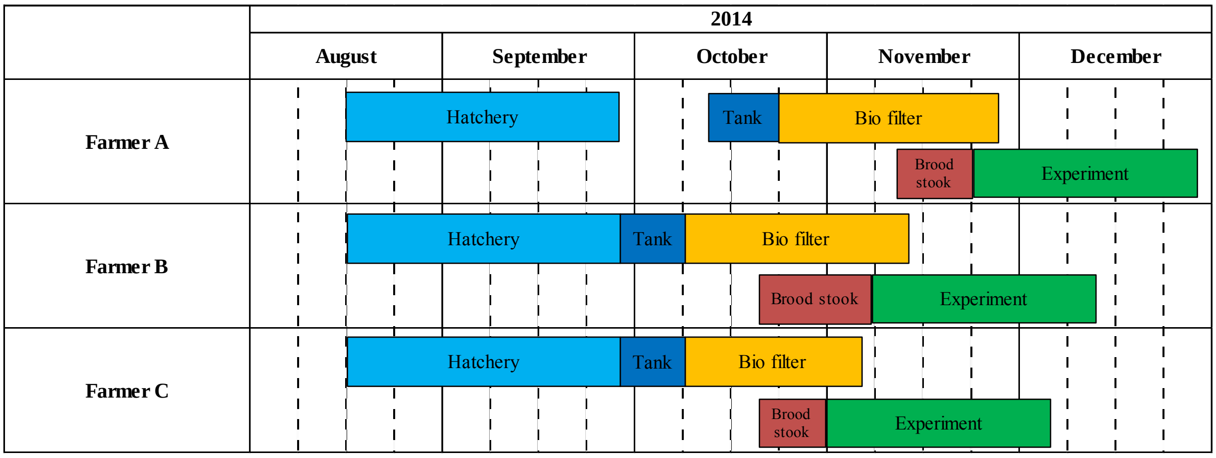
Table 5.1 Time schedule in this project study

	2014				
	August	September	October	November	December
Sites selection					
Hatchery preparation					
Tank preparation					
Bio filter preparation					
Broodstock management					
Experiment					
Harvest and analysis					

5.2 Schedule in each farmer

The experiment site is targeted 3 farmers. Experiment period for each farmer were displaced due to avoid the overlap for installation of equipment and introduce of method to farmer. That is, period of one farmer from installation to harvest is about 10 weeks and beginning period for each site is displaced 1-2 weeks (Table 5.2).

Table 5.2 Time schedule in each farmer



6 Target farmer selection and experimental group

6.1 Target farmer selection

Target province Takeo is located in south of Phnom Penh city. Takeo province is known as high potential area of natural giant fresh water prawn production to provide to Phnom Penh and other area. Takeo province is one of the target provinces by Fresh Water Aquaculture Improvement and Extension (FAIEX) project supported by JICA. Several seed production farmers supported by FAIEX has succeed the seed production of giant fresh water prawn since 2008.

In this project study, seed production farmers in this province were listed up as target farmers, and selected based on criteria. Seed production farmers list from FAIEX final report was reviewed and then some new farmer candidates were added for this project study (Table6.1).

Table 6.1 List of seed production farmer in Takeo province

No.	Name	Commune	District	Supported by FAIEX	
1	Van Po	Angtasom	Tramkak	○	2006
2	Prum Vat	Ankorborey	Ankorborey	○	2005
3	Duch Thol	Tropaingkronhong	Tramkak	○	2006
4	Long Yus	Tropaingthomkhangcheoung	Tramkak	○	2005
5	Ouch Heoun	Tropaingthomkhangtb0ng	Tramkak	○	2005
6	Men Hon	Ang Prasath	Kirivong	○	2005
7	Oung Phol	Nhengnhorng	Tramkak	○	2006
8	But Sreymom	Tangdong	Baty	○	2006
9	Nhorm Noy	Tramkak	Tramkak	○	2007
10	Ngov Lihour	Chomraspen	Samrong	○	2007
11	Pen Phea	Angkhnol	Trang	○	2007
12	Dong Sareoun	Pechsa	Kohandet	○	2007
13	Kheav Sam	Laybo	Tramkak	AIT	
14	Som Hak	Taphem	Tramkak	AIT	
15	Chhiv Nheng	Krangleav	Baty	AIT	
16	Vin Chheoun	Kus	Tramkak	AIT	
17	Soun Sothea	Outdomsorya	Tramkak	Volunteer	
18	Min Sao	Tropaingtrokeath	Tramkak	Volunteer	
19	Kong Sarean	Beungtranhkhangcheoung	Samrong	AIT+Seila	
20	Sean Phan	Angtasom	Tramkak	AIM	
21	Sem Phon	Ausaray	Tramkak	CT	
22	Hang Heang	Rokakhnong	Donkeo	Volunteer	
23	Khol Bol	Sophy	Baty	Volunteer	
24	Tes Salath	Cheangtong	Tramkak	GM	
25	Oum Thy	Popel	Tramkak	GM	
26	Sem Sorn	Samrong	Tramkak	GM	
27	Sot Sarorn	Srieronong	Tramkak	GM	
28	Trok Nhav	Rokakhnong	Donkeo	GM	
29	Nget Samoen	Ou Saray	Tramkok	Volunteer	

6.2 Selection policy and methods

Target farmer selection put the priority of farmers who are carrying out a giant fresh water prawn seed production or have experience in the past. Because there is prior information that some farmer could not carry out the experiment in individual situations, Selection is aimed to target 3 farmer in addition of survey target to seed production farmer without experience.

Table 6.2 Conditions to confirm for selection

	Condition
(1)	Farmer is operating the seed production of giant fresh water prawn or has experiment of production.
(1)’	Farmer carries on seed production in multiple fish species (including catfish) more than 5 year.*1
(2)	Farmer has animus to continue the seed production of giant fresh water prawn.
(3)	There is space for hatchery (green house)
(4)	Farmer can provide the labor (himself or worker) for experiment.
(5)	Vehicle for delivery of equipment can access during rainy season.
(6)	Ground water can be used.*2
(7)	Public electricity is present or not.*3

*1 Although initial plan was (1) is essential condition, from the fact that farmers who meet the conditions (1) and (2) was less than 3 farmers, condition was modified to add (1)’ .
*2 Aquaculture famer in rural area normally use reserve water in pond or river water for seed production. So that farmer has ground water is few. This survey was considered to transport the ground water from neighborhood.
*3 Solar panel installation site was priority selected no public power farmers.

6.3 Selection result

According to interviews in fisheries office of Takeo province, 4 farmers have the experience giant fresh water prawn seed production in the past and 1 farmer is carrying out now. However other 3 farmers have stopped it because there is no seed demand and it was not benefit-able. Querying further information about the seed production farmers who can meet the condition, department of aquaculture development and fisheries office in Takeo Province, recommended 3 more farmers aside from these, total 7 farmers were listed up.

Among these 7 farmers, 3 farmers don’t have intension of seed production or were not cooperative for project study, thus filed survey was implemented in remained 4 farmers. In consequence, Mr. Prum Vat (Farmer A), Mr. Hang Heang (Farmer B) and Mr. Van Po (Farmer C) have been selected as target farmers in this.

Table 6.3 Selection result

	Farmer name	(1)	(1)'	(2)	(3)	(4)	(5)	(6)	(7)	Remark	Result
A	Prum Vat	○	○	○	○	○	○	○	○	There is public electricity, but break down is occurring 5-10 time per month. Deputy chief of seed production network in Takeo province.	Selected
B	Hang Heang	×	○	○	○	○	○	△	○	There is public electricity, but break down is occurring 3-5 time per month. There are ground water and city water.	Selected
C	Van Po	×	○	○	○	○	○	×	×	There is not public electricity. Chief of seed production network in Takeo province.	Selected
	Nget Samoen	×	×	○	○	×	○	×	×	He has started the seed production from 2011. It is far between farmer house and aquaculture site	

6.4 Experimental group

This project study has introduced the sun dry salt to reduce the third water cost and the solar panel system to adapt the status of the power supply in Cambodia. Because these had been first trial in Cambodia, in order to diminish risk, the experiment group was planned as Table 6.4. Implementation ranking was decided relatively access is a good place from the town of Takeo (Dounkeo) and relatively high degree of difficulty such as solar panels installed farmers and solar salt use farmers. That is, it was stared from farmer C → farmer B → farmer in order.

Farmer A (Mr. Prum Vat) site in Angkor Borey district originally had been planning the operation with public electricity. But blackout was informed after installation of facility in this entire district during 20 October 2014 to 20 January 2015. By this information, experiment should been tried with two generators for 24 hour during 60-70 days in rotation.

Table 6.4 Target farmer and experiment plot

	Target farmer	Village	Commune	District	Experiment plot	Electricity	Remark
A	Prum Vat※1	Prey Sambo	Ankorborey	Ankorborey	The third water type C prepared in Japan	Public electricity + generator	Confirmation the seed produce by farmer level
B	Hang Heang	Chork	Rokakhnong	Donkeo	The third water type C prepared by local sun-dried salt	Public electricity + generator	Confirmation the seed produce by local sun-dried salt
C	Van Po	Ou Phot	Angtasom	Tramkak	The third water type C prepared in Japan	Solar system + generator	Confirmation the seed produce by Solar system

Figure 6.1 Location of experiment farmer

7 Hatchery preparation

7.1 Hatchery

A temperature inside hatchery has to be kept high during larvae rearing especially at night time, thus greenhouse hatcheries were constructed in all experiment places. In original plan, it should be constricted as simple hatchery with bamboo and wood frame and size was only experiment space about 6m x 7m. But, farmers have expected that they would use not only giant fresh water prawn seed production but also multi-purpose in future, the hatchery as 7m×8m-7m×12m has constructed bigger than original plan on the condition that the cost of additional materials and additional part was covered by farmers side.

7.2 Local and Japan procurement equipments

Large equipments such as 1.2kL FRP tank and coral sand, etc. of local procurement were transported to each farmer site. The main local procurement equipments are shown in Table 7.1.

Table 7.1 Equipment of local procurement

No.	Item	Quantity	Remark
1	FRP tank(size：1.2kL)	15 tanks (5 tanks ×3 farmers)	Viet Nam
2	FRP tank(size：0.6kL)	6 tanks (2 tanks×3 farmers)	Viet Nam
3	Coral sand	About 1,700kg(550kg in each farmer)	Viet Nam
4	industrial tool, reagents, stationery, glassware, net, bucket, pump, simple measurement kit, generators, etc.	1 set	

Japan procurement equipments delayed about 1 month and was arrived in 25 September 2014. These equipments were transported to each farmer site. The main japan procurement equipments are shown in Table 7.2.

Table 7.2 Equipment of Japan procurement

No.	Item	Quantity	Remark
1	Electronic balance	1 unit	Battery, Weighing capacity610g, Min.:0.01g
2	Compression balance	1 unit	Battery,-Weighing capacity60kg,Min.:5g
3	Biological microscope	3 units (1unit × 3 farmers)	Battery, 10-400 zooms
4	Circulation pump	4 units (1 unit × 3 farmers + spare)	Total pump head is more than 3.3m
5	Protein skimmer	3 units (1unit × 3 farmers)	Immersion type
6	Air pomp	3 units (1unit × 3 farmers)	Discharge rate is more than 40L/min
7	Seawater concentration refractometer	3 units (1unit × 3 farmers)	ATAGO Co., Ltd.
8	Nitrifying bacterium for third water	60L (20L×3 farmers)	Cultured by the third water
9	The third water	8kL	Mainly raw materials
10	Filter tank	3 units (1unit × 3 farmers)	PVC
11	Plankton net	1 roll (51m)	Mess size：229μm

Based on the lessons of the last year, filter tank was designed the 700 L volume for 3kL breeding tank. Design drawing of filter tank and wool box is shown in Figure 7.1 and 7.2. In addition, protein skimmer which has been verified the advantage by Okayama University of science was installed. This equipment can be made the bubble release by water pump and release organic in breeding tank (Figure 7.3).

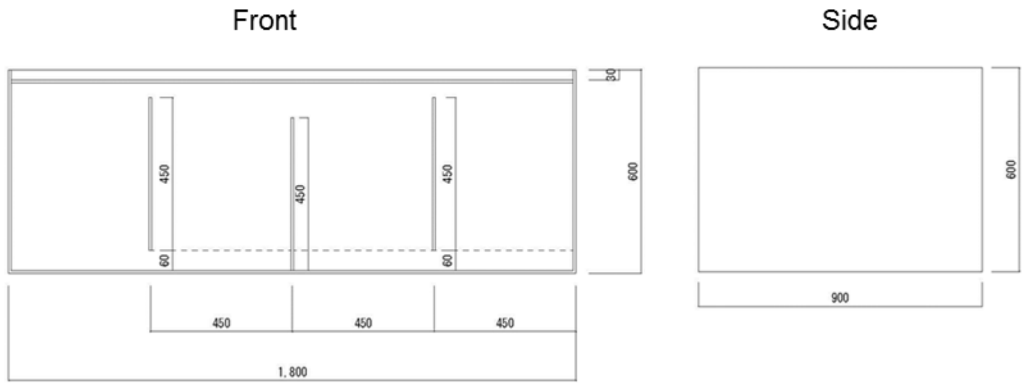


Figure 7.1 Design drawing of filter tank

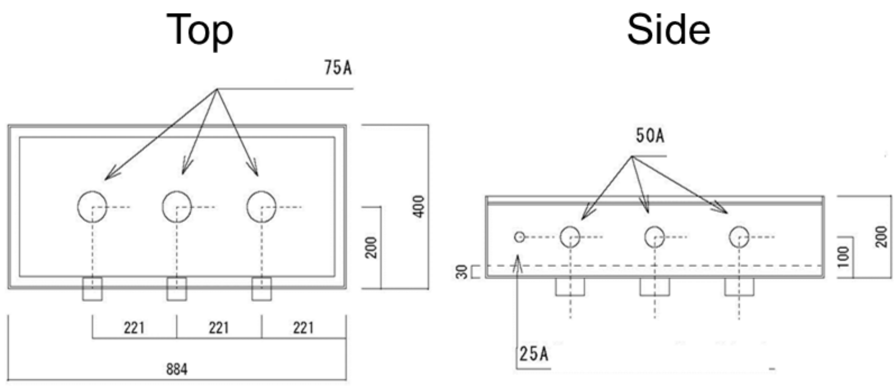


Figure 7.2 Design drawing of wool box

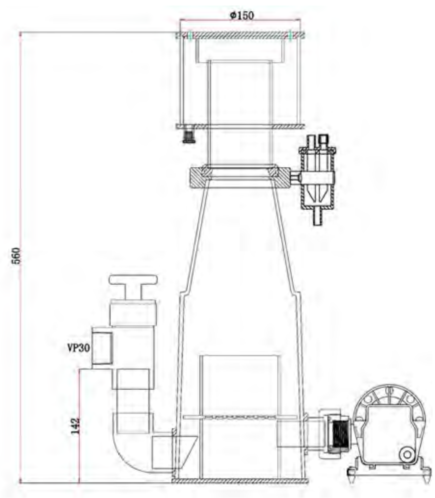


Figure 7.3 Structural drawing of protein skimmer
Reference: http://www.e-lss.jp/reefoctopus/products/p01_d_int.html

7.3 Solar panel system

Solar panel system has been installed in Farmer C (Mr. Van Po) site. Necessary electric power in this experiment is total 110W/hour of circulation pump (55W), air pump (25W) and protein skimmer (30W). And the safety factor in consideration of the inrush current as 1.8 safety factor. Solar panel system has been designed as 110W×24hr×1.8＝4800W/day. Main equipments of solar panel system are shown in Table 7.3.

Table 7.3 Main equipments of solar panel system

No.	Item	Specification	Quantity	Country of origin
1	Solar module	210Wp, 24V	8 units	Japan/EU
2	Battery	12V 100Ah	8 units	China
3	Inverter	1,200W-24V	1 set	Taiwan
4	Breaker	For battery and inverter	1 set	France

7.4 Experimental tanks

As the size of the hatchery changed from original plan, there is enough space. Thus layout of experimental tank and filter system was modified as follows.

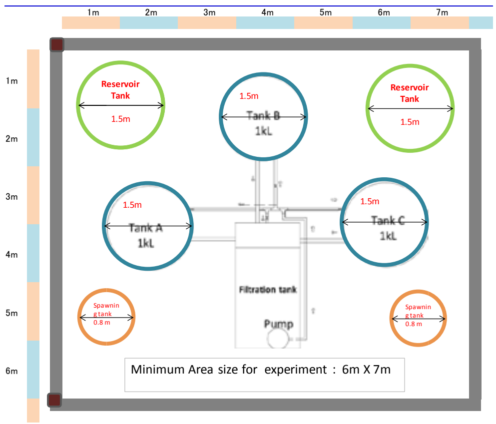


Figure 7.4 Original tank layout plan

Figure 7.5 Actual tank layout

8 Breeding water analysis and bio filter preparation

8.1 Breeding water

To check the locally available water whether it is applicable for breeding or not, the characteristics of the water such pH, electrical conductivity, calcium, magnesium and water hardness (bicarbonate ions) were measured in the groundwater and city water of each target farmer respectively. Dissolution of the third water ingredients were also tested whether it dissolves into each local water. As a result, third water ingredient does not dissolve completely into all the ground water, small amount of reminded sediment was observed in all cases. Later analysis has revealed that calcium ion in the third water combine with free bicarbonate ions and sediment was observed. On the other hand, third water ingredient dissolves into city water completely there was no remained any sediment in city water.

Table 8.1 Checking water in each farmer

	Farmer	Water source	pH	Electric conductivity (mS/m)	Ca (mg/L)	Mg (mg/L)	water hardness (dH)	Operating experience for Freshwater prawn	Sediment by the 3rd water
A	Prum Vat	Ground water	6.35	178.4	70	40	9.8	○	○
B	Hang Heang	City water	7.30	14.9	15	24	1	×	×
C	Van Po	Ground water	7.16	85.6	20	<1	22.4	×	○
	Kiv Sam	Ground water	7.12	81.0	—	—	—	○	○

Consequently city water was only one option to use in all experiment sites as breeding water. Breeding water of farmer A (Mr. Prum Vat) site was transported from water station in Angkorborey and farmer C (Mr. Van Po) site was transported from farmer B (Mr. Hang Heang) site.

Table 8.2 Breeding water in each farmer

	Farmer	Water source	Water supply
A	Prum Vat	City water	Transport from water station in Ankorborey
B	Hang Heang	City water	on site
C	Van Po	City water	Transport from Hang Heang site

8.2 Bio filter

After setting filter tank with coral sand, 700 L of breeding water filtered through (10μm) mesh bag was poured in filter tank. The breeding water was disinfected by free chlorine (50ppm) and it was neutralized by aeration for 24 hours. After then 7 L of nitrifying bacterium and 2 mg /L of ammonium chloride (NH_4Cl) was added in filter tank. The total ammonia (NH_3+ NH_4^+) and nitrite (NO_2^-) concentrations were measured almost every day. When each concentration becomes 0mg/L, Ammonium chloride was added again to the order of 3, 4, 5, 10, 15 and 20mg/L. Preparation period of the filter tank has been about 30 days, but were slightly back and forth at each site in relation to the larvae hatching date.

Breeding water circulation between filter tank and breeding tanks (1kL × 3 tanks) was initiated 6-7 days after the larvae breeding when umbrella artemia feeding is switched to the egg custard + artemia feeding. After starting circulation of breeding water, the filter tanks were maintained for total ammonia concentration to be 0mg / L and for the nitrite concentration to be 0-0.5mg / L as an indicator of appropriate water quality.

Table 8.3 Situation of filter tank preparation

	Prum Vat				Hang Heang				Van Po			
	Number of Day	Additive amount of ammonium chloride	Con.of NH_3+NH_4^+	Con. of NO_2^-	Number of Day	Additive amount of ammonium chloride	Con.of NH_3+NH_4^+	Con. of NO_2^-	Number of Day	Additive amount of ammonium chloride	Con.of NH_3+NH_4^+	Con. of NO_2^-
2014/10/8					Day-1	2mg/L	0mg/L		Day-1	2mg/L	0mg/L	
2014/10/9					Day-2				Day-2			
2014/10/10					Day-3				Day-3			
2014/10/11					Day-4				Day-4			
2014/10/12					Day-5	3mg/L	0mg/L	0mg/L	Day-5	3mg/L	0mg/L	0mg/L
2014/10/13					Day-6				Day-6			
2014/10/14					Day-7				Day-7			
2014/10/15					Day-8	4mg/L	0mg/L	0mg/L	Day-8	4mg/L	0mg/L	0mg/L
2014/10/16					Day-9				Day-9			
2014/10/17					Day-10				Day-10			
2014/10/18					Day-11	5mg/L	0mg/L	0mg/L	Day-11	5mg/L	0mg/L	0mg/L
2014/10/19					Day-12		0mg/L	0.5mg/L	Day-12		0mg/L	5mg/L
2014/10/20					Day-13	5mg/L	0mg/L	0mg/L	Day-13	5mg/L	0mg/L	0mg/L
2014/10/21	Day-1	2mg/L	0mg/L	2mg/L	Day-14		0mg/L	5mg/L	Day-14		0mg/L	5mg/L
2014/10/22	Day-2		0mg/L	2mg/L	Day-15	10mg/L	0mg/L	0mg/L	Day-15	10mg/L	0mg/L	0mg/L
2014/10/23	Day-3		0mg/L	0mg/L	Day-16		0mg/L	5mg/L	Day-16		0mg/L	5mg/L
2014/10/24	Day-4	3mg/L	0mg/L	0mg/L	Day-17		0mg/L	0mg/L	Day-17		0mg/L	0mg/L
2014/10/25	Day-5		0mg/L	0mg/L	Day-18	15mg/L	5mg/L	5mg/L	Day-18	15mg/L	0mg/L	0mg/L
2014/10/26	Day-6		0mg/L	0.2mg/L	Day-19		0mg/L	5mg/L	Day-19		2mg/L	5mg/L
2014/10/27	Day-7	4mg/L	0mg/L	0mg/L	Day-20		0mg/L	0mg/L	Day-20		0mg/L	2mg/L
2014/10/28	Day-8		0mg/L	5mg/L	Day-21	20mg/L	5mg/L	5mg/L	Day-21	20mg/L	0mg/L	0mg/L
2014/10/29	Day-9		0mg/L	0mg/L	Day-22		0mg/L	0.5mg/L	Day-22		2mg/L	5mg/L
2014/10/30	Day-10	5mg/L	0mg/L	0mg/L	Day-23		5mg/L	5mg/L	Day-23		0mg/L	0mg/L
2014/10/31	Day-11		0mg/L	5mg/L	Day-24	20mg/L	0mg/L	5mg/L	Day-24	20mg/L	0mg/L	0mg/L
2014/11/1	Day-12		0mg/L	0mg/L	Day-25		0mg/L	0mg/L	Day-25		2mg/L	5mg/L
2014/11/2	Day-13	10mg/L	0mg/L	0mg/L	Day-26		0mg/L	5mg/L	Day-26	20mg/L	0.5mg/L	5mg/L
2014/11/3	Day-14		0mg/L	5mg/L	Day-27	20mg/L	5mg/L	5mg/L	Day-27	20mg/L	0.5mg/L	5mg/L
2014/11/4	Day-15		0mg/L	1mg/L	Day-28		0mg/L	5mg/L	Day-28		0mg/L	5mg/L
2014/11/5	Day-16	15mg/L	0mg/L	0mg/L	Day-29		0mg/L	0mg/L		Water cycele start	0mg/L	0mg/L
2014/11/6	Day-17		0mg/L	5mg/L	Day-30	20mg/L	0mg/L	0mg/L			0mg/L	0mg/L
2014/11/7	Day-18		0mg/L	2mg/L	Day-31		0mg/L	5mg/L			0mg/L	0.5mg/L
2014/11/8	Day-19	20mg/L	0mg/L	5mg/L	Day-32	20mg/L	1mg/L	5mg/L			0mg/L	0mg/L
2014/11/9	Day-20		0mg/L	5mg/L	Day-33		0mg/L	5mg/L			0mg/L	0mg/L
2014/11/10	Day-21		0.5mg/L	0mg/L	Day-34	20mg/L	1mg/L	5mg/L			0mg/L	0mg/L
2014/11/11	Day-22	20mg/L	0mg/L	0mg/L	Day-35	20mg/L	0mg/L	5mg/L			0mg/L	0mg/L
2014/11/12	Day-23		0.5mg/L	1mg/L		Water cycele start	0mg/L	0mg/L			0mg/L	0mg/L
2014/11/13	Day-24		0mg/L	0.5mg/L			0mg/L	0mg/L			0mg/L	0mg/L
2014/11/14	Day-25	20mg/L	1mg/L	0.5mg/L			0mg/L	2mg/L			0mg/L	0mg/L
2014/11/15	Day-26		0mg/L	0.5mg/L			0mg/L	1mg/L			0mg/L	0mg/L
2014/11/16	Day-27		0mg/L	0mg/L			0mg/L	0.5mg/L			0mg/L	0mg/L
2014/11/17	Day-28	20mg/L	1mg/L	0mg/L			0mg/L	0.5mg/L			0mg/L	0mg/L
2014/11/18	Day-29		0mg/L	0.5mg/L			0mg/L	0.5mg/L			0mg/L	0mg/L
2014/11/19	Day-30	20mg/L	1mg/L	5mg/L			0mg/L	0.5mg/L			0mg/L	0mg/L
2014/11/20	Day-31		0mg/L	0.5mg/L			0mg/L	0.5mg/L			0mg/L	0mg/L
2014/11/21	Day-32	20mg/L	0mg/L	0mg/L			0mg/L	0.5mg/L			0mg/L	0mg/L
2014/11/22	Day-33						0mg/L	0.5mg/L			0mg/L	0mg/L
2014/11/23	Day-34	20mg/L					0mg/L	0.5mg/L			0mg/L	0mg/L
2014/11/24	Day-35	20mg/L	5mg/L	5mg/L			0mg/L	0.5mg/L			0mg/L	0mg/L
2014/11/25	Day-36	20mg/L	0mg/L	5mg/L			0mg/L	0.5mg/L			0mg/L	0mg/L
2014/11/26		Water cycele start	0mg/L	0mg/L			0mg/L	0.5mg/L			0mg/L	0mg/L
2014/11/27			0mg/L	0mg/L			0mg/L	0.5mg/L			0mg/L	0mg/L
2014/11/28			0mg/L	0.5mg/L			0mg/L	0mg/L			0mg/L	0mg/L
2014/11/29			0mg/L	0.5mg/L			0mg/L	0mg/L			0mg/L	0mg/L
2014/11/30			0mg/L	0.5mg/L			0mg/L	0mg/L			0mg/L	0mg/L

*Measurement of the total ammonia and nitrite concentrations using the test kit of Sera (measuring range is each 0.0-5.0mg/L).

*Fill part is period of circulation between filter tank and breeding tanks.

9 Broodstock management

9.1 Broodstock procurement

Broodstock with egg procurement carried out in giant fresh water prawn markets located north of Takeo city. However, there are minor of amount of broodstock with dark color egg (eyed egg) because this procurement time is from October to November near the end is the rainy season of reproduction period of giant fresh water prawn. In view of the time to carry to the second and third farmer, there is a risk that a shortage of the necessary amount of broodstock with egg. Therefore, it was decided to also the simultaneous purchase of broodstock with orange color eggs (non-eyed egg) to hatch after 1 week and these broodstocks were transported and stocked in fresh water in the second and third farmer (Table 9.1). And after hatching in each farmer, in case of broodstock with egg remaining, it was transported to next farmer.

Table 9.1 procurement schedule of broodstock with egg

Farmer	Experiment order	4th week on Oct.		1th week on Nov.		2th week on Nov.
Van Po	1	Broodstock with dark egg (eyed egg)	transport	↓		
Hang Heang	2	Broodstock with orange egg	keep →	Broodstock with dark egg (eyed egg)	transport	↓
Prum Vat	3			Broodstock with orange egg	keep →	Broodstock with dark egg (eyed egg)

Broodstocks with dark egg (eyed egg) are stocked in the 3rd water
Broodstocks with orange egg are stocked in fresh water

9.2 Hatching

Three breeding tanks of 1kL volume were prepared each site and then 60,000 larvae were stocked in each tank (180,000 larvae / 3 tanks / site). For the larval stage and for the day of switching the feed to be same schedule in terms of feed preparation and easy growth observation, larvae hatching-out in a same day have to be stocked at once in 3 breeding tanks each site.

When the water recirculation system with bio-filter tank was prepared, each site started stocking larvae respectively. Consequently hatching larvae was stocked in farmer C (Mr. Van Po) site on 29 and on 30 October, in farmer B (Mr. Hang Heang) site on 6 November, and in farmer A (Mr. Prum Vat) site on 20 and on 21 November.

As it is informed previously, number of berried female had been decreasing in November, moreover water temperature of broodstock tanks in a hatchery dropped to 26 degree, therefore quite less hatching were observed and it was difficult to obtain necessary number of larvae in farmer A (Mr. Prum Vat) site. As a countermeasure for less hatching, broodstock tanks covered by vinyl sheet were submerged into large concrete pond which could keep warm water temperature. As a result, enough number of larvae for the experiment was obtained.

Table 9.2 procurement result of broodstock with egg

Date	Prum Vat		Hang Heang		Van Po	
	Broodstock with Dark eggs in thak (head)	Larvae (head)	Broodstock with Dark eggs in thak (head)	Larvae (head)	Broodstock with Dark eggs in thak (head)	Larvae (head)
10/29					42	60,000
10/30					54	120,000
10/31						
11/1						
11/2			30	118,000		
11/3			30	49,000		
11/4			26	77,700		
11/5			25	11,000		
11/6			20	178,000		
11/7			9	125,400		
11/8-11/10						
11/11						
11/12	18	32,600				
11/13	28	11,460				
11/14	28	17,500				
11/15	31	67,000				
11/16	33	4,000				
11/17	34	40,000				
11/18		20,000				
11/19		37,000				
11/20	34	100,000				
11/21	25	60,000				
Average	29	38,956	23	93,183	48	90,000
Maximum	34	100,000	30	178,000	54	120,000
Minimum	18	4,000	9	11,000	42	60,000

*Orange frame is larvae stocked in breeding tanks

*Red frame is day kept broodstock rearing tanks warm

10 Experiment contents

10.1 Experiment method

Stocking density, feeding method for seed production and monitoring items, etc. were the same as last year. Experiment method is shown in Table 10.1.

Based on the results of the last year, feeding table for 30 days breeding was introduced to farmers (Table 10.1). This table should be modified when breeding date is changed by the growth rate.

Table10.1 Experiment method

Item	Method / Contents
Water	The third water type C from Japan and The third water type C from Cambodia
Tank volume	1kL tank ×3 tanks (breeding tank)＋700L water tank (Filter tank)
Power source	A public electricity or solar panel (spare generator for each site)
Accessories	Air pump, circulation pump, protein skimmer (with a pump), greenhouse
Target site	3 seed producing farm in Takeo province
Stocking density	60 larvae /liter (60,000 larvae / 1 tank)
Feeding	Artemia umbrella, Artemia and egg custard are fed. Amount of feeding is controlled by the growth and the water quality.
Monitoring item	The water quality (water temperature, DO, pH, salinity, ammonia, nitrous acid) and growth stage (observation by microscope)
Monitoring method	-Person to conduct monitoring : Farmer -A consultant and an advisory mission instruct the method -MARDeC staff and extension officer in Takeo take part in monitoring regularly
Harvesting method	Water conversion by a half of the breeding quantity of water is performed three days before the crop and calculate the number of Post larva (PL) by the wet weight method (calculate it from multiplication of PL numbers per gram and PL gross weight)
Result analysis method	Calculate a survival rate of PL and compare it with the last seed and sapling production results and previous ones which are past similar scale

Table 10.2 Feeding table in case of 30 days breeding

Day	Day0	Day1-5	Day6	Day7-8	Day9-10	Day11-13	Day14-15	Day16	Day17-19	Day20-23	Day24-26	Day27-29	Day30
Stage	I	I-V	V	VI	VII	VII-VIII	VIII	VIII-IX	IX	X-XI (PL)	≧PL	≧PL	Harvest
Artemia (g cyst/1000L)	0	5 (Umbrella)		5				6		7			0
Egg custard (g/1000L)			4	6	7	8	9	10		12	14	8	0
Artificial food (g/1000L)	0											3	0

11 Experiment result

11.1 Farmer A site Mr. Prum Vat

The third hatching zoea was stocked in this site. It was difficult to secure enough number of berried prawn in November. Therefore, experiment in this site started on 20 November about 1 week behind schedule. On 20 November, 50,000 zoea / kL were stocked in two tanks respectively although 10,000 zoea smaller than planned. On 21 November, 60,000 zoea / kL were stocked in another one tank.

Larvae had grown steadily at initial stage (Fig 11.1). Afterward some amount of mortality started also inactive feeding has been observed 16 to 17 days after hatching. Accidentally an incubation of artemia failed due to lack of aeration 17 days (on 7 December) consequently it caused lack of feeding from afternoon to next morning (on 8 December). This accident possibly influenced larvae rearing condition. Growth of larvae stagnated from 19 days (Table 11.1) also consecutive mortality of larvae recorded from 100 to 3,000 in a day. As the number of larvae could be estimated around 1000 by visual observation, larval rearing was stopped on 19 December (after hatch out 30 and 31 days). As a result, survival rates were 3.20%, 1.69%, and 1.16% respectively in each tank (Table 11.1).

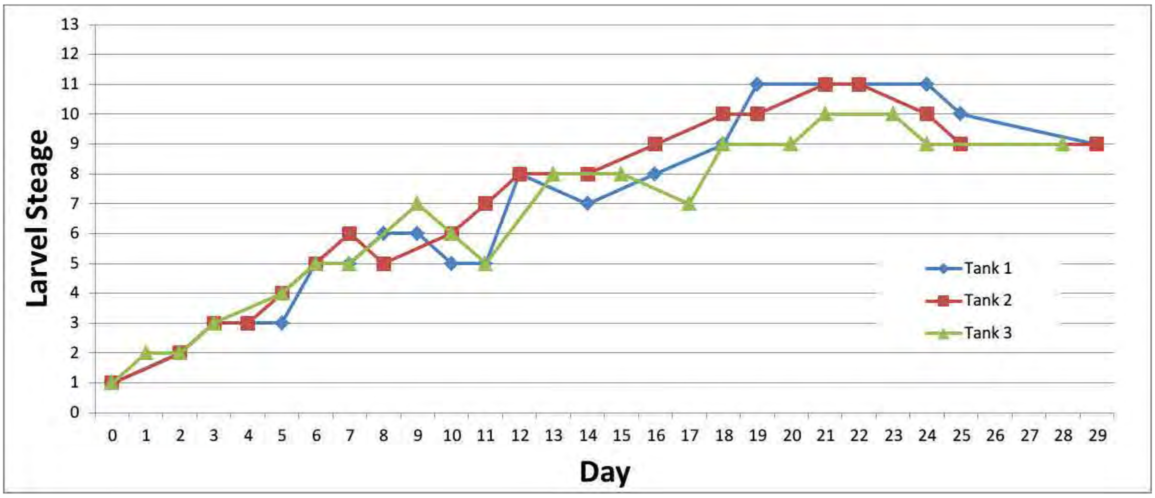


Figure 11.1 Transition of larval stage in each tank of Farmer A (Mr. Prum Vat) site
Growth rate was calculated that larval stage of I is 1, II is 2, III is 3….XI is 11, Pre-PL is 12 and PL is 13 in majority of larval stage.

Table 11.1 Result of harvest in farmer A (Mr. Prum Vat) site

	Day old	Start (zoea)	Harvest (zoea)	Survival rate (%)
TankNo,1	31	50,000	1,598	3.20
TankNo,2	31	50,000	846	1.69
TankNo,3	30	60,000	696	1.16
Total		160,000	3,140	1.96

Overview of water quality of each tank is shown in Table 11.2. And detail results are described in Annex 1-3. Water quality was kept normal in all measurement items including the total ammonia and nitrite. Floating and adhesion of algae was heavily raised in the rearing tank therefore water should be exchanged frequently as it was difficult to observe larvae in a tank.

Table 11.2 Result of water quality in farmer A (Mr. Prum Vat) site

		Temperature (℃)		DO (mg/L)	Salinity (psu)	pH	$NH_3+NH_4^+$ (mg/L)	NO_2^- (mg/L)
Time		9:00	16:00	9:00	9:00	9:00	9:00	9:00
Tank1	Minimum	26.0	30.0	6.64	12.0	7.12	0.00	0.00
	Maximum	31.0	34.0	7.92	14.0	8.19	0.50	0.50
	Average	29.6	31.1	7.15	12.8	7.43	0.02	0.15
Tank2	Minimum	26.0	30.0	6.71	12.0	7.18	0.00	0.00
	Maximum	31.0	34.0	9.38	14.0	8.39	0.00	0.50
	Average	29.6	31.2	7.37	12.9	7.49	0.00	0.16
Tank3	Minimum	25.5	30.0	6.59	12.0	7.25	0.00	0.00
	Maximum	31.0	34.0	9.11	14.0	8.49	0.00	0.50
	Average	29.4	31.4	7.31	12.9	7.56	0.00	0.10

After harvest, PL was not confirmed and larvae preferentially observed by stage IX in each tank (Table 13).

Table 11.3 Larval stage distribution table at the time of harvest in farmer A (Mr. Prum Vat) site

Stage	Tank1(n=108)		Tank2(n=108)		Tank3(n=103)	
VII	0	0%	2	2%	0	0%
VIII	5	5%	5	5%	6	6%
IX	41	38%	53	49%	55	53%
X	23	21%	28	26%	26	25%
XI	35	32%	18	17%	14	14%
Pre-PL	4	4%	2	2%	2	2%
Majority	IX		IX		IX	

11.2 Farmer B site Mr. Hang Heang

The second hatching zoea was stocked in this site. The experiment in this site started on 6 November with 60,000 zoea / kL in each of 3 tanks. Stagnation of growth in larvae was observed 10 days after stocking although remarkable mortality had not occurred yet. Afterward mass mortality was observed 17 to 19 days after stocking in each tank (Fig.11.2). Number of larvae was estimated 700-1,500 zoea by visual observation, larval rearing stopped on 3 December (26 and 27 days after hatching). As a result, survival rates were as 1.26% 1.56% and 0.76% respectively (Table 11.4).

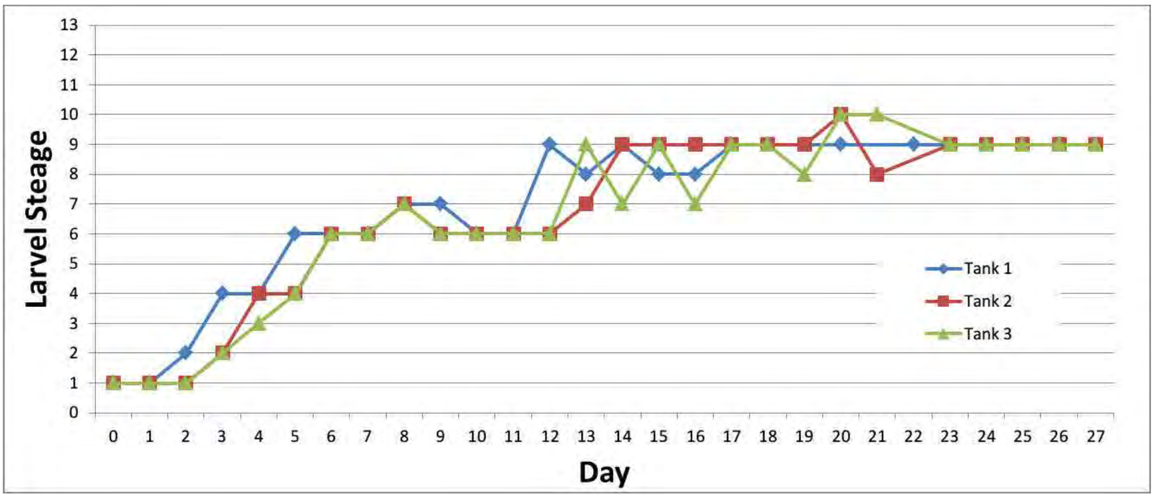


Figure 11.2 Transition of larval stage in each tank of farmer B (Mr. Hang Heang) site

Growth rate was calculated that larval stage of I is 1, II is 2, III is 3….XI is 11, Pre-PL is 12 and PL is 13 in majority of larval stage.

Table 11.4 Result of harvest in farmer B (Mr. Hang Heang) site

	Day old	Start (zoea)	Harvest (zoea)	Survival rate (%)
TankNo,1	26	60,000	756	1.26
TankNo,2	27	60,000	935	1.56
TankNo,3	27	60,000	456	0.76
Total		180,000	2,147	1.19

Only this site local sun dried salt produced in Kampot was used to prepare the third water. Local sun dried salt possibly contains a certain amount of impurities so that a lot of algae raised in rearing tank. Although water was exchanged frequently and clean the tank, outbreak of algae could not be prevented nevertheless. Overview of water quality of each tank is shown in Table 11.5. And detail results are described in Annex 4-6. For one time of after hatch out 7 and 8 days, nitrate became 2.0 mg / L. Otherwise, water quality was kept normal in all measurement items including the total ammonia and nitrite.

Table 11.5 Result of water quality in farmer B (Mr. Hang Heang) site

		Temperature (℃)		DO (mg/L)	Salinity (psu)	pH	NH_3+/NH_4^+ (mg/L)	NO_2^- (mg/L)
Time		9:00	16:00	9:00	9:00	9:00	9:00	9:00
Tank1	Minimum	28.0	30.0	6.37	12.0	7.01	0.00	0.00
	Maximum	31.0	34.0	7.68	14.0	7.46	0.50	2.00
	Average	30.1	31.8	7.06	13.5	7.19	0.13	0.37
Tank2	Minimum	28.0	30.0	6.61	12.0	7.02	0.00	0.00
	Maximum	32.0	33.0	7.26	14.0	7.64	0.50	2.00
	Average	30.0	31.5	7.00	13.6	7.27	0.11	0.34
Tank3	Minimum	28.0	30.0	6.39	12.0	6.59	0.00	0.00
	Maximum	31.0	34.0	7.37	14.0	7.69	0.50	2.00
	Average	30.0	31.6	6.96	13.6	7.21	0.11	0.38

After harvest, PL was not confirmed and nearly half of larvae in each tank was larval stage IX as well as farm A site (Table 11.6).

Table 11.6 Larval stage distribution table at the time of harvest in farmer B (Mr. Hang Heang)

Stage	Tank1(n=116)		Tank2(n=108)		Tank3(n=103)	
VIII	3	3%	1	1%	5	5%
IX	39	34%	48	44%	45	44%
X	18	16%	23	21%	13	13%
XI	33	28%	19	18%	25	24%
Pre-PL	23	20%	17	16%	15	15%
Majority	IX		IX		IX	

11.3 Farmer C site Mr. Van Po

The first hatching zoea was stocked in this site. 60,000 zoea / kL were stocked in one tank on 29 October and another 60,000 zoea / kL were stocked in two tanks respectively on 30 October. A preparation of artemia failed accidentally due to human error 7 days after hatching (on 5 November), consequently it caused lack of feeding from the evening to morning. But mortality and stagnation of growth were not observed like other 2 sites.(Figure 11.3) Harvest was carried out on 12 December (36 days and 37 days after hatching). As a result of harvest, survival rates were 55.14% 48.85% and 35.69% respectively (Table 11.7).

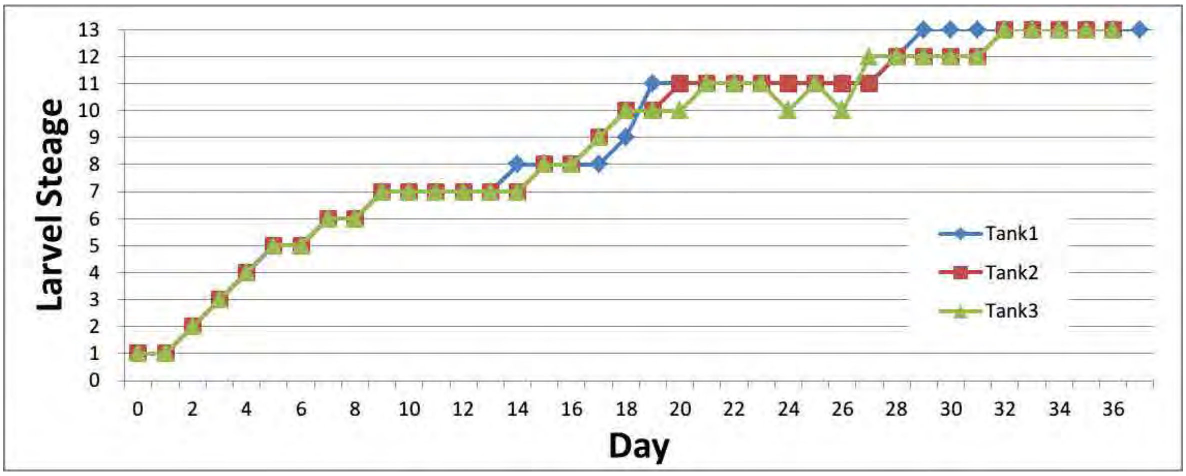


Figure 11.3 Transition of larval stage in each tank of farmer C (Mr. Van Po) site

Growth rate was calculated that larval stage of I is 1, II is 2, III is 3….XI is 11, Pre-PL is 12 and PL is 13 in majority of larval stage

Table 11.7 Result of harvest in farmer C (Mr. Van Po) site

	Day old	Start(zoea)	Harvest(PL)	Survival rate (%)	Transfer
TankNo,1	37	60,000	33,084	55.14	Intermediate rearing in tank
TankNo,2	36	60,000	29,310	48.85	Farmer B site
TankNo,3	36	60,000	21,411	35.69	Intermediate rearing in pond
Total		180,000	83,805	46.56%	

Overview of water quality of each tank is shown in Table 11.8. And detailed results are described in Annex 7-9. In this site, rearing water was not exchanged up to the harvest during 36 days as a deterioration of water quality was not observed.

Table 11.8 Result of water quality in farmer C (Mr. Van Po) site

		Temperature (℃)		DO (mg/L)	Salinity (psu)	pH	NH_3+NH_4^+ (mg/L)	NO_2^- (mg/L)
Time		9:00	16:00	9:00	9:00	9:00	9:00	9:00
Tank1	Minimum	29.0	30.0	6.40	12.0	6.83	0.00	0.00
	Maximum	31.0	33.0	6.81	13.0	7.55	0.50	0.50
	Average	29.9	31.6	6.63	12.7	7.32	0.04	0.01
Tank2	Minimum	29.0	30.0	6.36	12.0	7.14	0.00	0.00
	Maximum	31.0	32.0	6.85	13.0	7.53	0.50	0.50
	Average	29.9	31.6	6.67	12.2	7.37	0.04	0.01
Tank3	Minimum	29.0	30.0	6.44	12.0	7.10	0.00	0.00
	Maximum	31.0	32.0	6.87	13.0	7.71	0.50	0.50
	Average	29.9	31.6	6.69	12.2	7.35	0.04	0.01

11.4 Comparison of growth rate among 3 sites

Average of growth rate in each site was compared in Fig11.4. The farmer C (Mr. Van Po) site was temporarily slow down about 5 days in stage VII, after then larvae had been smoothly growth until PL. On the other hand, in farmer A (Mr. Prum Vat) and farmer B (Mr. Hang Heang) site, survival rate are about 1 %. Growth stage started varying from 7days and larvae finally stopped the growth in stage IX.

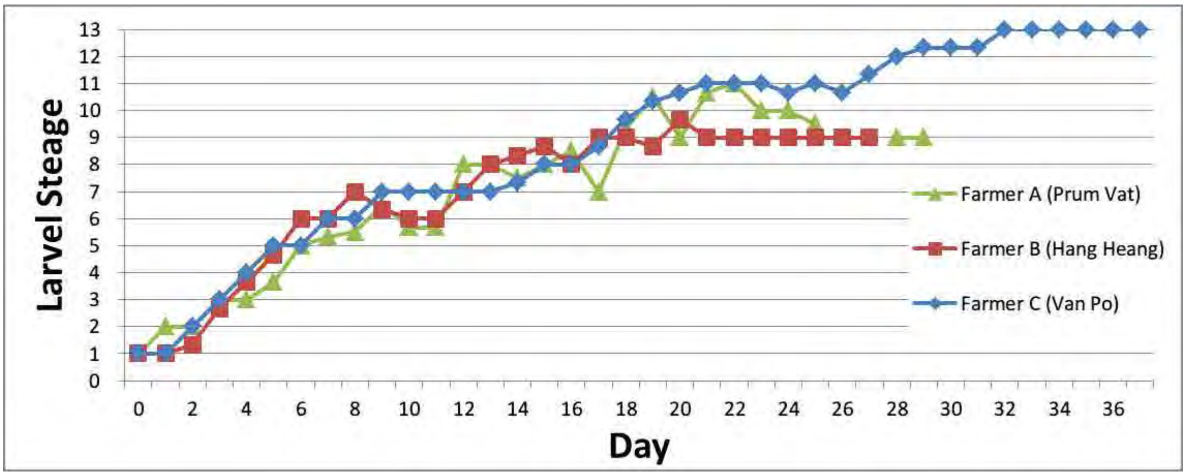


Figure 11.4 Transition of majority of larval stage in each farmer

12 Consequence analysis

12.1 Seed production cost and profitability

Price of raw material for the third water has been provisionally set 30 USD / kL as experimental sale price in Japan. When introducing in Cambodia, if 0.5USD/kL of city water is available, additional cost is not so large. However, cost of rearing water using the third water should be becomes 57.50 USD/kL (Table 12.1) in case city water should be transported from other place. The brine water (60 psu) can be transported in 50USD/kL from Kampot province to Takeo province consequently cost of rearing water (12 psu) diluted by ground water is 10USD/kL and it is cheaper than the third water.

On the other hand, it has been indicated the result that PL survival rate of the third water type C was 4.8 % higher than control (diluted seawater). Assuming production in average 46.6% of survival rate which the results of farmer C (Mr. Van Po) indicated, advantage of the third water was analyzed by comparing with the case of using the brine water.

Table 12.1 The third water and brine water cost and PL survival rate

Rearing water cost					PL survivl rate(%)
Type of water and cost		Diluted sea water		Cost per kL of 12 psu (USD)	
The third water (type C)	30 USD/kL	City water	0.5 USD/kL	30.5	46.6
		City water transported	27.5 USD/kL	57.5	46.6
		Ground water	NCW		
Brine water (60 psu)	50 USD/kL	Ground water	0 USD/kL	10.0	38.9

NCW: not complied with

(1) Seed production cost

In order to evaluate from the view of sustainability for giant fresh water seed production, initial cost of hatchery facility and equipment and operation cost such as consumables were investigated on the basis of expense record of this experiment and hearing from farmer and local distributer. Some materials imported were calculated by using foreign currency exchange rate, 1USD＝107.24 JPY and 1USD＝4,000 Riel (September 2014). Necessary equipment and operation cost for giant fresh water prawn seed production are shown (A)-(D) in Table 12.2.

Table 12.2 Necessary equipment and operation cost for giant fresh water prawn seed production

(A)Initial facility cost (only 1st year)

Item	Cost	Remark
Facility	2,400.0 USD/place	Simple Green House construction costs (calculated from this project study)
Tank	1,000.0 USD/set	3 rearing tanks and 2 water exchange tanks (calculated from this project study)
Filter tank	2,114.9 USD/pcs	Order made in Japan
Filter material (coral sand)	800.0 USD/1kL	Made in Vietnam (calculated from this project study)
Solar panel system*	8,095.0 USD / set	4,800wh / day、Battery with guarantee for 12 year

*In case of no public electricity only

(B)Hatchery facility operation cost (every year)

Item	Cost	Remark
Material for the third water type C	30.0 USD/kL	Nominal price (Experimental price)
City water	0.5 USD/kL	Water station (calculated from this project study)
Transportation of city water including water cost	27.5 USD/kL	In case of transport within 10km area
Transportation of brine water (60psu)	50.0 USD/kL	Kampot Province → Takeo Province (by interview) Diluted to 12 psu for rearing water
Equipment maintenance	240.0 USD	Every year spending about 10% of the construction costs, such as repair of damaged part

(C)Operating costs and consumables (every cycle)

Item	Cost	Remark
Electric bill	1.20 USD / day	0.25US$/kw/hr× 0.2kw× 24hr
Feed	90.6USD / 3kL rearing / cycle	Artemia：124.9USD/kg Egg custard：13.4USD/kg crumble (Powder feed)：1USD/kg
Broodstock	20.0 USD / kg	Natural broodstock with egg
Consumables	40.0 USD / cycle	Simple water quality test kit, beaker, etc.
labor	5.0 USD / day /person	Calculated from this project study

(D)Intermediate rearing cost (every cycle)

Item	Cost	Remark
Fertilization	10.0 USD / pond/time	0.25US$/kw/hr× 0.2kw× 24hr
Drainage and irrigation fee	20.0 USD / pond/time	Pump amortization or leased necessary to drainage and irrigation, and fuel cost
Consumables	20.0 USD / cycle	Netting, wood, rope, etc.
labor	5.0 USD / day /person	Calculated from this project study

(2) Assumption of famer model

Each farmer has different facility's condition such as availability of city water, ground water and public electricity etc. If considering the case of using the third water and the case of not using the third water, 10 models can be assumed in total as shown in Table 12.3. Revenue in each case is compared in case farmer will be continuing seed production in the first year and from the second year on.

Table 12.3 Case that is assumed for each conditions of seed production farmer

Case	The third water	City water	Transportation of city water	Public electricity	Solar panel	This experiment	PL Survival rate (up to 40 days)
1	Use	○	×	○	-	Farmer B (Hang Heang)	46.6%
2	Use	○	×	×	need		46.6%
3	Use	×	○	○	-	Farmer A(Prum Vat)	46.6%
4	Use	×	○	×	need	Farmer C (Van Po)	46.6%
5	Non use → use of brine water	○	×	○	-		41.8%
6	Non use → use of brine water	○	×	×	need		41.8%
7	Non use → use of brine water	×	○	○	-		41.8%
8	Non use → use of brine water	×	○	×	need		41.8%
9	Non use → use of brine water	Present of ground water	×	○	-		41.8%
10	Non use → use of brine water	Present of ground water	×	×	need		41.8%

○: present、×: absent

(3) Seed production cost in each model case

To estimate the total production cost from case 1 to 10 of farmer model, the necessary expenses of each items for seed production are summarized in Table 12.4 in each of the model.

Table 12.4 Expenses necessary to seed production (Unit: USD)

(A)Initial facility cost (only 1st year)			Case									
Item	Cost (USD)	Qty	1	2	3	4	5	6	7	8	9	10
Facility	2,400.00 USD/place	×1	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400	2,400
Tank	1,000.00 USD/set	×1	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
Filter tank	2,114.90 USD/pcs	×1	2,115	2,115	2,115	2,115	2,115	2,115	2,115	2,115	2,115	2,115
Filter material (coral sand)	800.00 USD/1kL	×1	800	800	800	800	800	800	800	800	800	800
Solar panel system*	8,095.00 USD / set	×1	0	8,095	0	8,095	0	8,095	0	8,095	0	8,095
Total (A)			**6,315**	**14,410**	**6,315**	**14,410**	**6,315**	**14,410**	**6,315**	**14,410**	**6,315**	**14,410**
(B)Hatchery facility operation cost (every year)			Case									
Item	Cost (USD)	Qty	1	2	3	4	5	6	7	8	9	10
Material for the third water type C	30.00 USD/kL	×3 kL	90	90	90	90	0	0	0	0	0	0
City water	0.50 USD/kL	×3 kL	1.5	1.5	0	0	1.5	1.5	0	0	0	0
Transportation of city water including water cost	27.50 USD/kL	×3 kL	0	0	82.5	82.5	0	0	82.5	82.5	0	0
Transportation of brine water (60psu)	50.00 USD/kL	×0.6 kL	0	0	0	0	30	30	30	30	30	30
Equipment maintenance	240 USD	×1	240	240	240	240	240	240	240	240	240	240
Total (B)			**332**	**332**	**413**	**413**	**272**	**272**	**353**	**353**	**270**	**270**
(C)Operating costs and consumables (every cycle)			Case									
Item	Cost (USD)	Qty	1	2	3	4	5	6	7	8	9	10
Electric bill(*1)	1.20 USD / day	×60days	72	0	72	0	72	0	72	0	72	0
Feed(*2)	90.60USD / 3kL breeding / cycle	×1 cycle	90.6	90.6	90.6	90.6	90.6	90.6	90.6	90.6	90.6	90.6
Broodstock(*3)	20.00 USD / kg	×1.5	30	30	30	30	30	30	30	30	30	30
Consumables	40.00 USD / cycle	×1 cycle	40	40	40	40	40	40	40	40	40	40
labor(*1)	5.0 USD / day /person	×45days	225	225	225	225	225	225	225	225	225	225
(D)Intermediate breeding cost (every cycle)			Case									
Item	Cost (USD)	Qty	1	2	3	4	5	6	7	8	9	10
Fertilization	10 USD / pond/time	×1	10	10	10	10	10	10	10	10	10	10
Drainage and irrigation fee(*4)	20 USD / pond/time	×1	20	20	20	20	20	20	20	20	20	20
Consumables	20.00 USD / cycle	×1	20	20	20	20	20	20	20	20	20	20
labor	5.0 USD / day /person	×40days	200	200	200	200	200	200	200	200	200	200
Total (C)+(D)			**707.6**	**635.6**	**707.6**	**635.6**	**707.6**	**635.6**	**707.6**	**635.6**	**707.6**	**635.6**

(*1) 60 days including preparation
(*2) Including crumble at the intermediate rearing
(*3) Considering the extraction rate is assumed to 1.5 times
(*4) Pump amortization or leased necessary to drainage and irrigation, and fuel cost

Production costs based on the above are shown in Table 12.5. In Cambodia, giant fresh water seed production may be practiced 3 times per year at maximum because natural berried prawn is available in limited period only from July to November.　Cost of 3 time production in first year with public electricity like the case 1, 3, 5, 7, and 9 is about 9,000USD and with solar panel system is about 17,000USD.　Subsequently 3 time production is continued in second year, cost is about 2,200 per year.

Table 12.5 Seed production cost of each production (Unit: USD)

Seed production cost		Case									
		1	2	3	4	5	6	7	8	9	10
1st year (A)＋(B)＋(C)＋(D)	Annual production times: 1 time	7,354	15,377	7,435	15,458	7,414	15,437	7,495	15,518	7,413	15,436
	Annual production times: 2 times	8,062	16,085	8,143	16,166	8,122	16,145	8,203	16,226	8,120	16,143
	Annual production times: 3 times	8,769	16,792	8,850	16,873	8,829	16,852	8,910	16,933	8,828	16,851
After 2nd year (B)＋(C)＋(D)	Annual production times: 1 time	1,039	967	1,120	1,048	1,099	1,027	1,180	1,108	1,098	1,026
	Annual production times: 2 times	1,747	1,603	1,828	1,684	1,807	1,663	1,888	1,744	1,805	1,661
	Annual production times: 3 times	2,454	2,238	2,535	2,319	2,514	2,298	2,595	2,379	2,513	2,297

(4)　Production number and sale revenue

Only farmer A (Mr. Prum Vat) has an experience of prawn seed sales in Takeo province. According to him, prawn seed (PL 30) can be sold at 300 Riel / head. Thus sales income of each farmer model is estimated based on this price.

Survival rate up to PL using the third water was 46.6 % that is average in farmer C (Mr. Van Po) in project study. And using the brine water was 41.8 that lower 4.8 points base on result in last year. Survival rate after intermediate breeding of 30 days assumed 60 % according to information from farmer who has experience and national aquaculture center in Cambodia.

Production number and sale revenue are shown in Table 12.6.

Table 12.6 Production number for sale and sale revenue of each production number

Production number for sale and sale revenue		Case									
		1	2	3	4	5	6	7	8	9	10
		The third water				Brine water					
Production number for sale (head)	Annual production times: 1 times	50,760				45,576					
	Annual production times: 2 times	101,520				91,152					
	Annual production times: 3 times	152,280				136,728					
Sale revenue (USD)	Annual production times: 1 times	3,807				3,418					
	Annual production times: 2 times	7,614				6,836					
	Annual production times: 3 times	11,421				10,255					

(5) Revenue presumption

Annual balances of each production case depend on the time of production. Sales revenue are shown in Table 12.7.

Table 12.7 Annual balance of each production number (Unit: USD)

Year	Nmber of production	Case									
		The third water				Brine water					
		1	2	3	4	5	6	7	8	9	10
Balance in 1st year	Annual production times: 1 time	-3,547	-11,570	-3,628	-11,651	-3,876	-11,899	-3,957	-11,980	-3,874	-11,897
	Annual production times: 2 times	-448	-8,471	-529	-8,552	-1,165	-9,188	-1,246	-9,269	-1,164	-9,187
	Annual production times: 3 times	2,652	-5,371	2,571	-5,452	1,545	-6,478	1,464	-6,559	1,547	-6,476
Balance after 2nd year	Annual production times: 1 time	2,768	2,840	2,687	2,759	2,439	2,511	2,358	2,430	2,441	2,513
	Annual production times: 2 times	5,867	6,011	5,786	5,930	5,150	5,294	5,069	5,213	5,151	5,295
	Annual production times: 3 times	8,967	9,183	8,886	9,102	7,860	8,076	7,779	7,995	7,862	8,078
the average of the production three times a year after 2nd year		Case 1-4		9,034		Case 5-10			7,942		

Case of 3 times production per year with public electricity in case of 1,3,5,7 and 9 was becomes positive earnings in the first year, about 2,600 USD of profit in using the third water and about 1,500 USD of profit in using the brine water. However, Case of 3 times production per year with solar panel system was becomes negative earnings of 5,000 to 6,500 USD in first year. After then, form the second year on, case of 3 times production per year using the third water in case of 1 to 4 is 9,034 USD on average and using brine water in case of 5 to 10 is 7,942 USD on average. Case of using the third water improved 14 % of profit. Total incomes of each model case form first year are shown in Table 12.8. In case of 2 times production per year with solar panel system, all of the case should be becomes negative earnings, but all of the case with solar panel system with 3 times production per year can be becomes positive earnings at the end of second year.

Table 12.8 Total income of each model case (Unit: USD)

Total income (1st year - 4th year)		Case									
		The third water				Brine water					
		1	2	3	4	5	6	7	8	9	10
At the end of 1st year	Annual production times: 1 time	-3,547	-11,570	-3,628	-11,651	-3,876	-11,899	-3,957	-11,980	-3,874	-11,897
	Annual production times: 2 times	-448	-8,471	-529	-8,552	-1,165	-9,188	-1,246	-9,269	-1,164	-9,187
	Annual production times: 3 times	2,652	-5,371	2,571	-5,452	1,545	-6,478	1,464	-6,559	1,547	-6,476
At the end of 2nd year	Annual production times: 1 time	-779	-8,730	-941	-8,892	-1,437	-9,388	-1,599	-9,550	-1,434	-9,385
	Annual production times: 2 times	5,420	-2,459	5,258	-2,621	3,985	-3,895	3,823	-4,057	3,988	-3,892
	Annual production times: 3 times	11,619	3,812	11,457	3,650	9,406	1,599	9,244	1,437	9,409	1,602

(6) Revenue compared with the conventional method

It is suggested a possibility that the giant fresh water prawn seed production could be profitable business model in case farmer has acquired technique to produce PL over average survival rate and continues 3 times production per year. Case of setting solar panel system also can be returned of investment and considerably improved revenue form third year on. In particular, case of having public electricity and using the third water is expected higher profit. The third water has been indicated superiority to compare with brine water as conventional method. Revenue single year balances from second year on without initial investment in A of Table 12.2 are shown in Table 12.9. It is expected increasing the revenue about 14 % by the third water. Furthermore, although this study could not be confirmed, it is expected additional savings by using the sun dry salt and groundwater.

Table 12.9 Revenue compared with the conventional method

(Single year balance from second year on)

Assumed case	Number of production		
	1 time/year	2 time/year	3 time/year
Public electricity (solar panel not required)			
Revenue (USD) of using the third water (Case 1 and 3)	2,727	5,827	8,926
Revenue (USD) of using the Brine water (Case 5, 7 and 9)	2,413	5,123	7,834
Increase rate of revenue rate	113%	114%	114%
No public electricity (solar panel required)			
Revenue (USD) of using the third water (Case 2 and 4)	2,799	5,971	9,142
Revenue (USD) of using the Brine water (Case 6, 8 and 10)	2,485	5,267	8,050
Increase rate of revenue rate	113%	113%	114%

12.2 Sun dry salt

The third water prepared by sun dry salt from Kampot province was used in farmer B (Mr. Hang Heang) site. In this site, mass mortality was observed 17 to 19 days after hatching and PL could not be harvested. The sun dry salt was analyzed and checked component like growth inhibitor (Table 12.10).

Iron (Fe), I measured cadmium (Cd), zinc (Zn), copper (Cu), lead (Pb), potassium (K), calcium (Ca), magnesium (Mg) and water content in sun dry salt were analyzed and iron and lead like metal detected except the main component of salt is potassium, calcium and magnesium. If these metals are detected high concentration, it is possible can be influenced as toxic to giant fresh water prawn larvae. But, in this experiment, final concentration of these metals for the third water which diluted the sun dry salt were that iron was less than 0.01 mg /L and lead was less than 0.06 mg / L. Especially lead has been known to be toxic to aquatic organisms, Acutely Toxic Effect test for PL of giant fresh water prawn was shown half lethal dose was 10mg / L when exposed for 96 hours[2]. However knowledge of chronic toxicity by lead in the larval stage of giant fresh water is absence. The results of the analysis for rearing water of farmer B using sun dry salt and farmer C using pure salt from Japan was 0.3 mg / L and 0.4 mg / L, rearing water of farmer C harvested PL was higher than another one. These results, it is considered a mass mortality occurred in farmer B is not derived from sun dry salt.

Table 12.10 Result of analysis for sun dry salt in Kampot province

Fe	Cd	Zn	Cu	Pb	K	Ca	Mg	water
ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	%
0.6	<0.25	<0.25	<0.5	5	2423	1660	6338	8.61

12.3 City water

In farmer A (Mr. Prum Vat) site also, mass mortality was confirmed from hatch out 16 days and PL could not be harvest. One of the different between farmer A and farmer C (Mr. Van Po) is city water for rearing water.

To examine the quality of city water, iron (Fe), cadmium (Cd), zinc (Zn), copper (Cu), lead (Pb), were analyzed (Table 12.11)

As a result, there was no difference except the lead. As mentioned above, lead is known to be toxic to aquatic organisms. However, in the concentration of city water that farmers A was used (0.2mg / L), it is unlikely that showed toxicity against giant fresh water prawn larvea.

[2] Fafioye, O.O. and 2Ogunsanwo, B.M (2007):The comparative toxicities of cadmium, copper and lead to Macrobrachium rosenbergii and Penaeusmonodon postlarvae, African Journal of Agricultural Research

Table 12.11 Result of analysis for city water used in this experiment

sample	Fe mg/L	Cd mg/L	Zn mg/L	Cu mg/L	Pb mg/L
City water in farmer B Mr. Han g Heang	＜0.1	＜0.05	＜0.05	＜0.1	<0.1
City water in farmer A Mr. Prum Vat	＜0.1	＜0.05	＜0.05	＜0.1	0.2

12.4 Ground water

In this project study, it has revealed that calcium ion in the third water combine with free bicarbonate ions and sediment was observed. Analysis of the heavy metals of the ground water, a high concentration of values in all the items were not detected (Table 12.12). Therefore,

If free bicarbonate ions can be removed in advance, there is a possibility that ground water can be used as water for mixing the raw materials of the third water. As removal method that can be used in Cambodia, (1) a powerful aeration (more than 80L / min) put in ground water about 1 week and (2) ground water is adjusted by hydrochloric acid to pH 7 and aeration (80L / min) about 1 day. And then although requires equipment, it can be used as (3) reverse osmosis membrane type water purifier. In this project study, method of (2) by acetic acid was tried and confirmed ground water can be used for the third water. It is possible to save the transportation cost of city water in the region there is no city water.

Table 12.12 Result of analysis for ground water

Sample	Fe mg/L	Cd mg/L	Zn mg/L	Cu mg/L	Pb mg/L
Ground water in farmer C Mr. Van Po	＜0.1	＜0.05	0.07	＜0.1	<0.1
Ground water in farmer B Mr. Hang Heang	＜0.1	＜0.05	＜0.05	＜0.1	0.6
Ground water in farmer A Mr. Prum Vat	＜0.1	＜0.05	＜0.05	＜0.1	<0.1

12.5 Disease

In late stage of larvae in giant fresh water prawn, white tail disease has been reported in Thailand and Viet Nam etc. but has not been reported in Cambodia. This disease is caused by *M.rosenbergii* Nodavirus (*Mr*NV) and extra small virus (XSV). When it is occurred, mortality reaches about 90 %. In order to confirm, the virus inspection or RT-PCR (reverse transcription polymerase chain reaction) test is required. Primers for RT-PCR and primers to increase rising detection sensitivity for nested PCR have been published by the OIE (Office International des Epizooties) (Table 12.13).

Table 12.13 Primer sequence for detecting the white tail disease

Primer	Reaction	Size	Sequence
For *Mr*NV			
*Mr*NV Forward	PCR	425bp	5'-GCGTTATAGATGGCACAAGG-3'
*Mr*NV Reverse			5'-AGCTGTGAAACTTCCACTGG-3'
*Mr*NV Forward	Nested PCR	205bp	5'-GATGACCCCAACGTTATCCT-3'
*Mr*NV Reverse			5'-GTGTAGTCACTTGCAAGAGG-3'
For XSV			
XSV Forward	PCR	546bp	5'-CGCGGATCCGATGAATAAGCGCATTAATAA-3'
XSV Reverse			5'-CCGGAATTCCGTTACTGTTCGGAGTCCCAA-3'
XSV Forward	Nested PCR	236bp	5'-ACATTGGCGGTTGGGTCATA-3'
XSV Reverse			5'-GTGCCTGTTGCTGAAATACC-3'

OIE (2012)

In order to confirm the reason of the mass mortality of larvae in farmer A (Mr. Prum Vat), samplings were carried out on 13 December and 19 December (harvest) in each tank and analyzed by RT-PCR. Primers were procured from Japan and analysis was carried out in MARDeC. And samplings were carried out the PL in farmer C (Mr. Van Po) and the PL were put in tank of farmer A in 6 days for infection experiment and the egg, swimming leg and muscle of broodstock to confirm the vertical infection. Target samples and result are shown in Table 12.14 and Fig. 12.1 and 12.2.

Table 12.14 Target samples and result for white tail disease

	Sample	*M.rosenbergii* Nodavirus(*Mr*NV)	Extra small Virus(XSV)
	Zoea＆PL		
1	Prum Vat1 (Zoea) sampling in 13 Dec.		
2	Prum Vat2 (Zoea) sampling in 13 Dec.		
3	Prum Vat3 (Zoea) sampling in 13 Dec.		
4	Prum Vat1 (Zoea) sampling in 19 Dec.	●	
5	Prum Vat2 (Zoea) sampling in 19 Dec.	●	
6	Prum Vat3 (Zoea) sampling in 19 Dec.	●	
7	Van Po(PL before transportation) sampling in 14 Dec.		
8	Van Po(PL after transportation) sampling in 19 Dec.	●	
	Broodstock		
9	Broodstock1 (Egg)	●	
10	Broodstock1 (Leg)	●	
11	Broodstock1 (Muscle)		
12	Broodstock2 (Egg)		
13	Broodstock2 (Leg)		
14	Broodstock2 (Muscle)		
15	Broodstock3 (Egg)		
16	Broodstock3 (Leg)	●	
17	Broodstock3 (Muscle)		

● is positive

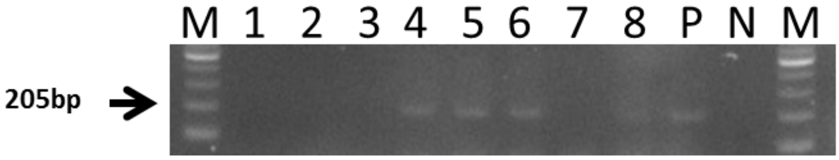


Figure 12.1 Result of nested RT-PCR for *Mr*NV in zoea and PL

M : 100bp marker、1: Prum Vat1 (Zoea) sampling in 13 Dec., 2: Prum Vat2 (Zoea) sampling in 13 Dec., 3: Prum Vat3 (Zoea) sampling in 13 Dec., 4: Prum Vat1 (Zoea) sampling in 19 Dec., 5: Prum Vat2 (Zoea) sampling in 19 Dec.、6: Prum Vat3 (Zoea) sampling in 19 Dec., 7: Van Po(PL before transportation) sampling in 14 Dec., 8: Van Po(PL after transportation) sampling in 19 Dec., P:Positive control, N:Negative control

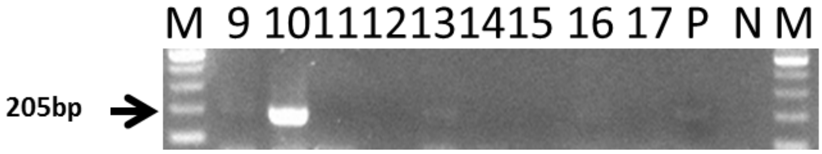


Figure 12.2 Result of nested RT-PCR for *Mr*NV in broodstock

M : 100bp marker, 9:Broodstock1 (Egg), 10:Broodstock1 (Leg), 11:Broodstock1 (Muscle), 12:Broodstock2 (Egg), 13:Broodstock2 (Leg), 14:Broodstock2 (Muscle), 15:Broodstock3 (Egg), 16:Broodstock3 (Leg), 17:Broodstock3 (Muscle), P:Positive control, N:Negative control

The results of this analyzed, Positive reaction were confirmed in larvae of farmers A site sampling on 19 December, PL of farmers C after transport to farmer A tank, and egg and swimming leg of broodstock.

It has the possibility of vertical infection in this experiment because positive reaction was detected 2 of 3 broodstocks though this sample is not same individual for this experiment. However, sample on 13 December was negative reaction. This result is considered possible was below the detection limit at the 13 December, but this disease is not first cause for the mass mortality, virus carrier larvae below the detection limit by RT-PCR has an onset of disease around 19 December by some sort of stress.

Since the disease is still in the research stage, the main cause has not been identified, and medicine and measures has not been established also. In addition, disinfection method for egg and larvae is not present. Possible defense method is to diagnose the broodstock by RT-PCR and broodstock has negative reaction is used to seed production. But farmer level is impossible.

In Thailand, although this disease has been frequently reported up to 10 years ago, reported cases of the disease has decreased because seed production farmer has been stopped to use the natural broodstock and carried out the broodstock management. In order to reduce the risk of this disease, the brood stock management of giant fresh water prawn would be require in Cambodia also.

12.6 Other potential mortality factors

As mass mortality occurrence factor, possibility of the sun dry salt, city water and disease were analyzed. However, direct factor cannot be detected. The other factors is possible of mortality in this experiment are indicated the below. It is considered one of the mortality factors by direct or consequential for trigger of disease and/or growth inhibiter.

(1) Electricity

Farmer C (Mr. Van Po) site could be harvest PL has been installed the solar panel system and water recycling filter system and aeration are consistently working. On the other hand, Farmer A (Mr.Prum Vat) and farmer B(Mr. Hang Heang) was occurred mass mortality were used generator and public electricity. It has confirmed generator and public electricity are not stabilized. Although the handling is easy to the output changes and blackout in daytime, it is difficult when these occur in nighttime. It is considered that lack of oxygen by shutoff the aeration is occurred in nighttime.

(2) Temporary food shortage

In farmer A (Mr. Prum Vat) and farmer C (Mr. Van Po), artemia culture was not operated by lack of aeration and lack of feed was occurred form afternoon to morn. In particular, farmer A site, mortality was began at next day of lack of feed. Although it is temporary, it t is considered the possibility that lack of feed given the stress to larvae.

(3) Egg quality

In generally, natural broodstock of giant fresh water prawn is caught in spawning period from July to October in Cambodia and it has known that good quality larvae should be obtained in this time. However, broodstock obtained from end of October and November was used in this experiment. Degradation of egg quality is known from latter period of spawning in some fish species[3]. Egg quality using this experiment was not better than it in spawning period. Especially egg quality of farmer A (Mr. Prum Vat) and farmer B (Mr. Hang Heang) were occurred mass mortality might be influenced to larvae rearing. According to farmer A who is carrying out giant fresh water prawn seed production, the survival rate is sometime low in case of use egg after spawning period.

[3] Shingo Watanabe (2004) : Relationship between spawning period of brood scorpion fish, *Sebastiscus marmoratus* in their breeding season and survival at early stage of their larvae in seed production

13 Consideration and discussion

13.1 Practicality of the third water and water recirculating filter system at the farmer level

Although only farmer C (Mr. Van Po) site which is using the solar panel system and the third water type C could harvest PL, in terms of technic and scale, it is confirmed in one instance that seed production of giant fresh water prawn is possible by introducing the water recycling filter system and the third water without transportation of seawater and water exchange.

By using seed production system designed as saving energy and efficient in this experiment, it has been confirmed that seed production by seawater could be possible in a country without sea, inland and certain area without electricity.

It is suggested a possibility to establish the giant fresh water prawn seed production business model in case experienced farmer can produce PL stably over average survival rate and continues 3 times production per year. Case of setting up solar panel system also can be returned of investment and considerably improved revenue form third year on. Furthermore, although this study could not be confirmed, it is expected additional savings by using the sun dry salt and groundwater. This is fundamental to save the cost for aquaculture.

By using seed production system designed as saving energy and efficient in this experiment, it has been confirmed that seed production by seawater could be possible in a country without sea, inland and certain area without electricity.

Although this project study was targeting giant fresh water prawn seed production, white shrimp (*P. vannamei*) culture carried out in south Asia had been implemented with the third water by Okayama University of science. As a result, it has been obtained higher performance that a harvest amount was 1.53kg / m^2 and a survival rate was 61 % and, it is also expected to verify in developing countries.

13.2 Extension of giant fresh water prawn seed production technology

Mr. Hang Heang and Mr. Van Po are currently making plan of seed production for next year by using same production facility as this experiment. On the other hand, in Takeo province there exist even experienced farmers who have stopped prawn seed production with reason of no needs and benefits according to the information at target farmer selection. Moreover from past experience of aquaculture extension in Cambodia, it is necessary to take care not only for seed production but also for downstream such as intermediate breeding, aquaculture technology and feed supply system so that each production segment can be connected in order to promote entire production cycle of giant fresh water prawn. Although there still remain several challenges that need to be addressed for the extension for giant fresh water prawn seed production technology, it is also considered there is some amount of needs for seed because 2 farmers already ordered PL that is harvested in farmer C site.

3 farmers who were targeted in this project study are seed production farmers that have been brought up by FAIEX, JICA technical cooperation project. They have enough experience and high

implementation capacity of training to extend aquaculture technology and it is also place to buy fingerling for aquaculture. It is expected that farmers who participated in this project study would continue the seed production as well as would promote prawn production technology because they recognize that customer to come to buy fingering will increase if more farmer knows prawn production technique. During larval rearing in this project study there was chance for seed producing farmers of FAIEX phase 2 to visit farmer C (Mr. Van Po). And then some farmers from Battambang and Siem Rea province are interested in the water recirculating system as these 2 provinces are located far from the sea. The target farmers of this project study could show another new option of aquaculture to existing seed farmers.

Annex1 Monitoring result in tank 1 in farmer A (Mr. Prum Vat) site

Date	Larvae		Water exchange (L)	Water quality							Substrate
	Day	stage/number		Temperature (℃)		DO (mg/L)	Salinity (psu)	pH	$NH_3+NH_4^+$ (mg/L)	NO_2^- (mg/L)	
				09:00	16:00	09:00	16:00	09:00	09:00	09:00	
11/20	0	I/10									
11/21	1										
11/22	2	II/10			33.0				0.00	0.00	
11/23	3	II/1III/9		31.0		7.08	13	7.36	0.00	0.00	
11/24	4	III/10		31.0	32.0	7.05	13	7.36	0.00	0.00	
11/25	5	III/10		31.0	34.0		13		0.00	0.00	
11/26	6	V/10		31.0	32.0		13		0.50	0.00	
11/27	7	V/8VI/2		31.0	31.0				0.00	0.50	
11/28	8	V/1VI/9		31.0	31.0				0.00	0.50	
11/29	9	VI/7VII/3		31.0	31.0						
11/30	10	V/6VII/2VIII/2		30.0	31.0	7.00		7.20	0.00	0.50	
12/1	11	V/8VI/1VIII/1		30.0	31.0				0.00	0.50	
12/2	12	VI/3VII/1VIII/6		29.0	31.0				0.00	0.50	
12/3	13			30.0	31.0				0.0	0.5	
12/4	14	VI/1VIII/2VIII/7	250	30.0	31.0	6.64	14	7.12	0.00	0.50	
12/5	15			30.0	31.0		13		0.00	0.00	
12/6	16	VII/2VIII/6IX/2	500	29.0	31.0				0.00	0.50	
12/7	17			31.0	31.0				0.00	0.00	
12/8	18	IX/6X/3Pre-PL/1		31.0	31.0				0.00	0.00	
12/9	19	VI/1VII/1IX/1X/2XI/5		29.0			12		0.00	0.00	
12/10	20			28.0	30.0		12		0.00	0.00	
12/11	21	IX/2X/2XI/5Pre-PL/1		29.0	31.0	7.21		7.34	0.00	0.00	
12/12	22	IX/2X/2XI/4Pre-PL/2		30.0					0.00	0.00	
12/13	23						12		0.00	0.00	
12/14	24	X/3XI/5Pre-PL/2							0.00	0.00	
12/15	25	IX/3X/6XI/1	350	28.0	30.0				0.00	0.00	
12/16	26			27.0	30.0				0.00	0.00	
12/17	27			27.0	30.0	7.92		8.19	0.00	0.00	
12/18	28		650	26.0					0.00	0.00	
12/19	29										
Minmum				26.00	30.00	6.64	12.00	7.12	0.00	0.00	
Maximum				31.00	34.00	7.92	14.00	8.19	0.50	0.50	
Average				29.63	31.14	7.15	12.78	7.43	0.02	0.15	

Annex2 Monitoring result in tank 2 in farmer A (Mr. Prum Vat) site

Date	Larvae		Water exchange (L)	Water quality							Substrate
	Day	stage/number		Temperature (℃)		DO (mg/L)	Salinity (psu)	pH	$NH_3+NH_4^+$ (mg/L)	NO_2^- (mg/L)	
				09:00	16:00	09:00	16:00	09:00	09:00	09:00	
11/20	0	I/10									
11/21	1										
11/22	2	II/10			33.0				0.00	0.00	
11/23	3	II/2III/8		31.0					0.00	0.00	
11/24	4	III/10		31.0	32.0	7.03	13	7.35	0.00	0.00	
11/25	5	IV/10		31.0	34.0		13		0.00	0.00	
11/26	6	V/10		31.0	32.0		13		0.00	0.00	
11/27	7	VI/4VI/4		31.0	31.0				0.00	0.50	
11/28	8	V/6VI/3VII/1		30.0	31.0				0.00	0.50	
11/29	9			30.0	31.0				0.00	0.50	
11/30	10	V/5VI/5		30.0		7.04		7.31	0.00	0.50	
12/1	11	VI/3VII/5VIII/2		30.0	31.0				0.00	0.50	
12/2	12	VI/2VII/2VIII6		29.0	31.0	6.80		7.18	0.00	0.50	
12/3	13			30.0	31.0				0.00	0.50	
12/4	14	VI/2VIII/5IX/3	250	30.0	31.0	6.71	14	7.28	0.00	0.50	
12/5	15			30.0	31.0		13		0.00	0.00	
12/6	16	VII/2VIII/4IX/4	500	29.0	31.0				0.00	0.50	
12/7	17			31.0	31.0				0.00	0.00	
12/8	18	VI/2IX/3X/4XI/1		31.0	31.0				0.00	0.00	
12/9	19	IX/1X/1X/6Pre-PL/2		29.0					0.00	0.00	
12/10	20			29.0	30.0		12		0.00	0.00	
12/11	21	VII/1IX/2XI/5Pre-Pl/2		29.0	31.0	7.23		7.45	0.0	0.0	
12/12	22	VII/1IX/1X/2XI/4Pre-PL/2		30.0					0.00	0.00	
12/13	23						12		0.00	0.00	
12/14	24	IX/4X/5XI/1							0.00	0.00	
12/15	25	VII/1IX/6X/1XI/1Pre-PL/1	350	28.0	30.0				0.00	0.00	
12/16	26			27.0	30.0				0.00	0.00	
12/17	27			27.0		9.38		8.39	0.00	0.00	
12/18	28		650	26.0					0.00	0.00	
12/19	29								0.00	0.00	
Minmum				26.00	30.00	6.71	0.67	7.18	0.00	0.00	
Maximum				31.00	34.00	9.38	14.00	8.39	0.00	0.50	
Average				29.58	31.21	7.37	11.33	7.49	0.00	0.16	

Annex3　Monitoring result in tank 3 in farmer A (Mr. Prum Vat) site

Date	Larvae		Water exchange (L)	Water quality							Substrate
	Day	stage/number		Temperature (℃)		DO (mg/L)	Salinity (psu)	pH	$NH_3+NH_4^+$ (mg/L)	NO_2^- (mg/L)	
				09:00	16:00	09:00	16:00	09:00	09:00	09:00	
11/21	0	I/10									
11/22	1	I/2II/8							0.00	0.00	
11/23	2	I/2II/8			33.0		13		0.00	0.00	
11/24	3	II/3III/7		31.0	32.0	7.05	13	7.39	0.00	0.00	
11/25	4			31.0	34.0		13		0.00	0.00	
11/26	5	IV/10		31.0	32.0		13		0.00	0.00	
11/27	6	V/10		31.0					0.00	0.00	
11/28	7	IV/1V/7VI/2		30.0					0.00	0.50	
11/29	8										
11/30	9	V/5VII/5				6.95		7.35			
12/1	10	V/2VI/6VII/2		30.0	31.0				0.00	0.50	
12/2	11	V/6VI/2VII/2		29.0	31.0	6.78		7.25	0.00	0.50	
12/3	12			30.0	31.0				0.00	0.00	
12/4	13	VI/2VII/3VIII/4IX/1	250	30.0	31.0	6.59	14	7.35	0.00	0.50	
12/5	14			30.0	31.0		13		0.00	0.00	
12/6	15	VI/2VII/2VIII/4IX/2	500	29.0	32.0				0.00	0.50	
12/7	16			30.0					0.00	0.00	
12/8	17	VI/4VII/5IX/1		31.0	31.0				0.00	0.00	
12/9	18	VI/2VII/3IX/4X/1		29.0			12		0.00	0.00	
12/10	19										
12/11	20	VII/4VIII/1IX/5		29.0	31.0	7.40		7.54	0.00	0.00	
12/12	21	VII/2VIII/2IX/2X/3Pre-PL/1		30.0					0.00	0.00	
12/13	22						12		0.00	0.00	
12/14	23	VIII/1IX/4X/4XI/1		30.0					0.00	0.00	
12/15	24	IX/5X/3XI/2	350						0.00	0.00	
12/16	25			27.0	30.0				0.00	0.00	
12/17	26			27.0	30.0	9.11		8.49	0.00	0.00	
12/18	27		650	26.0					0.00	0.00	
12/19	28			25.5					0.00	0.00	
Minmum				25.50	30.00	6.59	0.67	7.25	0.00	0.00	
Maximum				31.00	34.00	9.11	14.00	8.49	0.00	0.50	
Average				29.36	31.43	7.31	11.52	7.56	0.00	0.10	

Annex4 Monitoring result in tank 1 in farmer B (Mr. Hang Heang) site

Date	Larvae		Water exchange (L)	Water quality							Substrate
	Day	stage/number		Temperature (℃)		DO (mg/L)	Salinity (psu)	pH	$NH_3+NH_4^+$ (mg/L)	NO_2^- (mg/L)	
				09:00	16:00	09:00	16:00	09:00	09:00	09:00	
11/7	0	I/10		30.0	31.0	7.14	14	7.09	0.00	0.00	
11/8	1	I/8II/2		30.0	31.5		14		0.00	0.00	
11/9	2	II/10		30.0	32.0		14		0.50	0.00	
11/10	3	III/1IV/9		31.0	32.0	6.37	14	7.13	0.50	0.00	
11/11	4	IV/10		31.0	33.0		14		0.50	0.00	
11/12	5	VI/10		30.0	31.0		14		0.50	0.00	
11/13	6	VI/10		30.0	30.0	6.55	14	7.01	0.50	0.00	
11/14	7	VI/10		30.0			14		0.00	2.00	
11/15	8	VII/10		30.0	32.0	6.76	14	7.46	0.00	0.50	
11/16	9	VII/10		30.0	32.0		14		0.00	0.50	
11/17	10	VI/10		29.0	30.0		14		0.00	0.50	
11/18	11	VI/10		28.0			14		0.00	0.50	
11/19	12	VII/2IX/5VIII/3		29.0			14		0.00	0.50	
11/20	13	VI/2VII/5VIII/2IX/1		28.0	30.0	7.68	14	7.28	0.00	0.50	
11/21	14	VII/2IX/8	60	29.0	32.0		13		0.00	0.50	
11/22	15	VII/3VIII/6IX/1		31.0	32.0				0.00	0.50	
11/23	16	VII/2VIII/6IX/2		31.0	33.0	7.44		7.36	0.00	0.50	
11/24	17	VIII/5IX/5		31.0	34.0		12		0.00	0.50	
11/25	18	VIII/3IX/6		31.0	34.0		12		0.00	0.50	
11/26	19	X/4XI/6		31.0	32.0	7.18	12	7.06	0.00	0.50	
11/27	20	VII/2IX/7Pre-PL/1	300	31.0	31.0	7.14	12	7.23	0.50	0.50	
11/28	21			31.0	32.0	6.96	12	7.22	0.00	0.50	
11/29	22	X/2XI/8		31.0	31.0				0.00	0.00	
11/30	23	X/1XI/8Pre-PL/1		30.0	32.0				0.00	0.50	
12/1	24			30.0	31.0				0.00	0.00	
12/2	25	IX/1X/3XI/4Pre-PL2		29.0	32.0	7.37		7.09	0.50	0.50	
12/3	26			30.0					0.00	0.00	
Minmum				28.0	30.0	6.37	12.00	7.01	0.00	0.00	
Maximum				31.0	34.0	7.68	14.00	7.46	0.50	2.00	
Average				30.1	31.8	7.06	13.45	7.19	0.13	0.37	

Annex5 Monitoring result in tank 2 in farmer B (Mr. Hang Heang) site

Date	Larvae		Water exchange (L)	Water quality							Substrate
	Day	stage/number		Temperature (℃)		DO (mg/L)	Salinity (psu)	pH	NH_3+NH_4^+ (mg/L)	NO_2^- (mg/L)	
				09:00	16:00	09:00	16:00	09:00	09:00	09:00	
11/6	0	I/10					14		0.00	0.00	
11/7	1	I/10		30.0	31.0	7.17	14	7.47	0.00	0.00	
11/8	2	I/8II/2		29.0	31.0		14		0.00	0.00	
11/9	3	II/10		30.0	32.0		14		0.50	0.00	
11/10	4	III/1,IV/9		30.0	32.0	6.73	14	7.02	0.50	0.00	
11/11	5	IV/10		30.0	32.0		14		0.50	0.00	
11/12	6	VI/10		30.0	31.0		14		0.50	0.00	
11/13	7	VI/10		30.0	30.0	6.61	14	7.09	0.50	0.00	
11/14	8	VII/10		30.0	30.0		14		0.00	2.00	
11/15	9	VI/10		32.0	32.0	6.83	14	7.44	0.00	0.50	
11/16	10	VI/10		30.0	32.0		14		0.00	0.50	
11/17	11	VI/10		29.0	30.0		14		0.00	0.50	
11/18	12	VI/10		28.0			14		0.00	0.50	
11/19	13	VI/2VII/5IX/3		29.0			14		0.00	0.50	
11/20	14	VII/3IX/7		28.0	30.0	7.12	14	7.64	0.00	0.50	
11/21	15	IX/9X/1	60	29.0	32.0		13		0.00	0.50	
11/22	16	VI/3VII/3IX/4		31.0	32.0				0.00	0.50	
11/23	17	VI/2VII/2VIII/3IX/3		31.0		7.26		7.29	0.00	0.50	
11/24	18	VII/1VIII/4IX/5		31.0	33.0		13		0.00	0.50	
11/25	19	VIII/5IX/5		31.0			12		0.00	0.50	
11/26	20	X/6XI/4		31.0	32.0	7.00	12	7.16	0.00	0.50	
11/27	21	VI/1VIII/4IX/2Pre-PL/3	300	31.0	31.0	7.11	12	7.28	0.50	0.50	
11/28	22			31.0	32.0	6.88		7.18	0.00	0.50	
11/29	23	IX/9XI/1		31.0	32.0				0.00	0.00	
11/30	24	VIII/1IX/3XI/2		30.0	32.0				0.00	0.50	
12/1	25	IX/8X/2		30.0	31.0				0.00	0.00	
12/2	26	IX/5X/1XI/2Pre-PL/1		29.0	32.0	7.26		7.11	0.00	0.00	
12/3	27			30.0					0.00	0.00	
Minmum				28.0	30.0	6.61	0.67	7.02	0.00	0.00	
Maximum				32.0	33.0	7.26	14.00	7.64	0.50	2.00	
Average				30.0	31.5	7.00	12.98	7.27	0.11	0.34	

Annex6 Monitoring result in tank 3 in farmer B (Mr. Hang Heang) site

Date	Larvae		Water exchange (L)	Water quality							Substrate
	Day	stage/number		Temperature (℃)		DO (mg/L)	Salinity (psu)	pH	$NH_3+NH_4^+$ (mg/L)	NO_2^- (mg/L)	
				09:00	16:00	09:00	16:00	09:00	09:00	09:00	
11/6	0	I/10					14		0.00	0.00	
11/7	1	I/10		30.0	31.0	7.08	14	7.27	0.00	0.00	
11/8	2	I/8,II/2		29.0	31.0		14		0.00	0.00	
11/9	3	II/10		31.0	32.0		14		0.50	0.00	
11/10	4	III/2,IV/8		30.0	32.0	6.65	14	7.01	0.50	0.00	
11/11	5	IV/10		30.0	32.0		14		0.50	0.00	
11/12	6	VI/10		30.0	31.0		14		0.50	0.00	
11/13	7	VI/10		30.0	32.0	6.39	14	6.59	0.50	0.00	
11/14	8	VII/10		30.0	30.0		14		0.00	2.00	
11/15	9	VI/10		30.0	32.0	6.79	14	7.42	0.00	0.50	
11/16	10	VI/10		30.0	32.0		14		0.00	0.50	
11/17	11	VI/10		29.0	30.0		14		0.00	0.50	
11/18	12	VI/10		28.0			14		0.00	0.50	
11/19	13	VII/3IX/7		29.0			14		0.00	0.50	
11/20	14	VII/5VIII/4IX/1		28.0	30.0	7.37	14	7.69	0.00	0.50	
11/21	15	VII/1IX/9	60	29.0	32.0		13		0.00	0.50	
11/22	16	VI/2VII/5VIII/2IX/1		31.0	32.0		13		0.00	0.50	
11/23	17	VII/3VIII/5IX/2		31.0		7.25		7.30	0.00	0.50	
11/24	18	VII/4IX/6		31.0	33.0		13		0.00	0.50	
11/25	19	VII/2VIII/5IX/3		31.0	34.0		12		0.00	0.50	
11/26	20	X/6XI/4		31.0	32.0	6.90	12	7.18	0.00	0.50	
11/27	21	IX/4X/5/Pre-PL/1	300	31.0	31.0	6.97	12	7.23	0.50	1.00	
11/28	22			31.0	32.0	6.92		7.22	0.00	0.50	
11/29	23	IX/4X/3XI/3		31.0	31.0				0.00	0.00	
11/30	24	IX/5XI4Pre-PL/1		30.0	32.0				0.00	0.00	
12/1	25	IX/7XI/2Pre-PL/1		30.0	31.0				0.00	0.50	
12/2	26	IX/7X/1XI/1Pre-PL/1		29.0	32.0	7.27		7.22	0.00	0.50	
12/3	27			30.0					0.00	0.00	
Minmum				28.0	30.0	6.39	0.67	6.59	0.00	0.00	
Maximum				31.0	34.0	7.37	14.00	7.69	0.50	2.00	
Average				30.0	31.6	6.96	12.98	7.21	0.11	0.38	

Annex7 Monitoring result in tank 1 in farmer C (Mr. Van Po) site

Date	Larvae		Water exchange (L)	Water quality							Substrate
	Day	stage/number		Temperature (℃)		DO (mg/L)	Salinity (psu)	pH	$NH_3+NH_4^+$ (mg/L)	NO_2^- (mg/L)	
				09:00	16:00	09:00	16:00	09:00	09:00	09:00	
10/29	0	I/10									
10/30	1	I/10		31.0	32.0				0.0	0.0	
10/31	2	II/10		30.0	32.0				0.0	0.0	
11/1	3	III/10		30.0	30.0	6.57	12	7.30	0.0	0.0	
11/2	4	IV/10		30.0	31.0		12		0.0	0.0	
11/3	5	V/10		30.0	30.0		12		0.5	0.0	
11/4	6	V/10		29.0	31.0		12		0.5	0.0	
11/5	7	VI/10		29.0	31.0		12		0.5	0.0	
11/6	8	VI/10		31.0	33.0		12		0.0	0.0	
11/7	9	VII/10		30.0	31.0	6.69	12	7.52	0.0	0.5	
11/8	10	VII/10		30.0	32.0		12		0.0	0.0	
11/9	11	VII/10		30.0	32.0		12		0.0	0.0	
11/10	12	VII/10		30.0	32.0	6.78	12	7.55	0.0	0.0	
11/11	13	VII/10		30.0	32.0		12		0.0	0.0	
11/12	14	VIII/10		30.0	32.0		12		0.0	0.0	
11/13	15	VIII/10		30.0	32.0	6.73	12	7.53	0.0	0.0	
11/14	16	VIII/10		30.0	32.0		12		0.0	0.0	
11/15	17	VIII/10		30.0	32.0	6.67	12	7.52	0.0	0.0	
11/16	18	IX/10		30.0	32.0		12		0.0	0.0	
11/17	19	XI/10		30.0	32.0		12		0.0	0.0	
11/18	20	XI/10		29.0	32.0		12		0.0	0.0	
11/19	21	XI/10		29.0	31.0		12		0.0	0.0	
11/20	22	XI/10		29.0	31.0	6.48	12	7.42	0.0	0.0	
11/21	23	XI/10		30.0	32.0		12		0.0	0.0	
11/22	24	X/3XI/5Pre-PL/2		31.0	32.0		12		0.0	0.0	
11/23	25	XI/7Pre-PL/3		30.0	32.0	6.50	12	7.14	0.0	0.0	
11/24	26	XI/8Pre-PL/2		30.0	32.0		12		0.0	0.0	
11/25	27	XI/7Pre-PL/3		31.0	32.0		13		0.0	0.0	○
11/26	28	XI/3Pre-PL/7		30.0	32.0	6.40	13	7.09	0.0	0.0	○
11/27	29	XI/2PL/8		30.0	31.0		13		0.0	0.0	○
11/28	30	XI/1PL/9		30.0	32.0	6.40	13	6.83	0.0	0.0	○
11/29	31	PL/10		30.0	32.0		13		0.0	0.0	○
11/30	32	PL/10		30.0	32.0		13		0.0	0.0	○
12/1	33	PL/10		30.0	31.0		13		0.0	0.0	○
12/2	34	PL10	320	30.0	31.0	6.81	10	7.12	0.0	0.0	○
12/3	35	PL10	500	29.0	31.0		6		0.0	0.0	○
12/4	36	PL10	600	29.0	31.0		3		0.0	0.0	○
12/5	37	PL10		29.0	31.0		3		0.0	0.0	○
Minmum				29.00	30.00	6.40	12.00	6.83	0.00	0.00	
Maximum				31.00	33.00	6.81	13.00	7.55	0.50	0.50	
Average				29.89	31.59	6.60	12.23	7.30	0.04	0.01	

Annex8 Monitoring result in tank 2 in farmer C (Mr. Van Po) site

Date	Larvae		Water exchange (L)	Water quality							Substrate
	Day	stage/number		Temperature (℃)		DO (mg/L)	Salinity (psu)	pH	NH_3+NH_4^+ (mg/L)	NO_2^- (mg/L)	
				09:00	16:00	09:00	16:00	09:00	09:00	09:00	
10/30	0	I/10		31.0	32				0.00	0.00	
10/31	1	I/10		30.0	32.0				0.00	0.00	
11/1	2	II/10		30.0	32.0	6.85	12	7.32	0.00	0.00	
11/2	3	III/10		30.0	31.0		12		0.00	0.00	
11/3	4	IV/10		30.0	30.0		12		0.00	0.00	
11/4	5	V/10		30.0	30.0		12		0.50	0.00	
11/5	6	V/10		29.0	31.0		12		0.50	0.00	
11/6	7	VI/10		30.0	32.0		12		0.50	0.00	
11/7	8	VI/10		30.0	31.0	6.80	12	7.53	0.00	0.50	
11/8	9	VII/10		30.0	32.0		12		0.00	0.00	
11/9	10	VII/10		30.0	32.0		12		0.00	0.00	
11/10	11	VII/10		30.0	32.0	6.75	12	7.51	0.00	0.00	
11/11	12	VII/10		30.0	32.0		12		0.00	0.00	
11/12	13	VII/10		30.0	32.0		12		0.00	0.00	
11/13	14	VII/10		30.0	32.0	6.70	12	7.53	0.00	0.00	
11/14	15	VIII/10		30.0	32.0		12		0.00	0.00	
11/15	16	VIII/10		30.0	32.0	6.71	12	7.50	0.00	0.00	
11/16	17	IX/10		30.0	32.0		12		0.00	0.00	
11/17	18	X/10		30.0	32.0		12		0.00	0.00	
11/18	19	X/10		29.0	32.0		12		0.00	0.00	
11/19	20	XI/10		29.0	31.0	6.66	12	7.42	0.00	0.00	
11/20	21	XI/10		30.0	32.0		12		0.00	0.00	
11/21	22	XI/10		31.0	32.0		12		0.00	0.00	
11/22	23	X/4XI/4Pre-PL/2		31.0	32.0		12		0.00	0.00	
11/23	24	X/3XI/5Pre-PL/2		30.0	32.0	6.50	12	7.2	0.00	0.00	
11/24	25	X/3XI/6Pre-PL/1		30.0	32.0		12		0.00	0.00	
11/25	26	X/1XI/5Pre-PL/4		31.0	32.0		13		0.00	0.00	
11/26	27	XI/8Pre-PL/2		30.0	32.0		13		0.00	0.00	
11/27	28	X/2XI/2Pre-PL/6		30.0	31.0		13		0.00	0.00	
11/28	29	X/1XI/3Pre-PL/6		30.0	32.0	6.36	13	7.18	0.00	0.00	
11/29	30	X/1XI/2Pre-PL/7		30.0	32.0		13		0.00	0.00	○
11/30	31	X/1XI/3Pre-PL/6		30.0	32.0		13		0.00	0.00	○
12/1	32			30.0	31.0		13		0.00	0.00	○
12/2	33	PL/10	320	30.0	31.0	6.73	10	7.14	0.00	0.00	○
12/3	34	PL/10	500	29.0	31.0		6		0.00	0.00	○
12/4	35	PL/10	600	29.0	31.0		3		0.00	0.00	○
12/5	36	PL/10	0	29.0	31.0		3		0.00	0.00	○
Minmum				29.00	30.00	6.36	0.67	7.14	0.00	0.00	
Maximum				31.00	32.00	6.85	13.00	7.53	0.50	0.50	
Average				29.95	31.62	6.67	11.86	7.37	0.04	0.01	

Annex9　Monitoring result in tank 3 in farmer C (Mr. Van Po) site

Date	Larvae		Water exchange (L)	Water quality							Substrate
	Day	stage/number		Temperature (℃)		DO (mg/L)	Salinity (psu)	pH	$NH_3+NH_4^+$ (mg/L)	NO_2^- (mg/L)	
				09:00	16:00	09:00	16:00	09:00	09:00	09:00	
10/30	0	I/10		31.0	32.0				0.00	0.00	
10/31	1	I/10		30.0	32.0				0.00	0.00	
11/1	2	II/10		30.0	32.0	6.78	12	7.10	0.00	0.00	
11/2	3	III/10		31.0	31.0		12		0.00	0.00	
11/3	4	IV/10		30.0	30.0		12		0.00	0.00	
11/4	5	V/10		29.0	31.0		12		0.50	0.00	
11/5	6	V/10		29.0	31.0		12		0.50	0.00	
11/6	7	VI/10		30.0	32.0		12		0.50	0.00	
11/7	8	VI/10		30.0	31.0	6.87	12	7.33	0.00	0.50	
11/8	9	VII/10		30.0	32.0		12		0.00	0.00	
11/9	10	VII/10		30.0	32.0		12		0.00	0.00	
11/10	11	VII/10		30.0	32.0	6.77	12	7.71	0.00	0.00	
11/11	12	VII/10		30.0	32.0		12		0.00	0.00	
11/12	13	VII/10		30.0	32.0		12		0.00	0.00	
11/13	14	VII/10		30.0	32.0	6.62	12	7.55	0.00	0.00	
11/14	15	VIII/10		30.0	32.0		12		0.00	0.00	
11/15	16	VIII/10		30.0	32.0	6.66	12	7.35	0.00	0.00	
11/16	17	IX/10		30.0	32.0		12		0.00	0.00	
11/17	18	X/10		30.0	32.0		12		0.00	0.00	
11/18	19	X/10		29.0	32.0		12		0.00	0.00	
11/19	20	X/10		29.0	31.0		12		0.00	0.00	
11/20	21	XI/10		29.0	31.0	6.71	12	7.29	0.00	0.00	
11/21	22	XI/10		30.0	32.0		12		0.00	0.00	
11/22	23	IX/2X/4XI/4		31.0	32.0		12		0.00	0.00	
11/23	24	X/6XI/3Pre-PL/1		30.0	32.0	6.6	12	7.3	0.00	0.00	
11/24	25	X/4XI/4Pre-PL/2		30.0	32.0		12		0.00	0.00	
11/25	26	X/5XI/3Pre-PL/2		31.0	32.0		13		0.00	0.00	
11/26	27	XI/3Pre-PL/7		30.0	31.0		13		0.00	0.00	
11/27	28	X/3XI/2Pre-PL/5		30.0	31.0		13		0.00	0.00	
11/28	29	X/2XI/3Pre-PL/5		30.0	32.0	6.44	13	7.27	0.00	0.00	
11/29	30	X/1XI/3Pre-PL/6		30.0	32.0		13		0.00	0.00	○
11/30	31	X/1XI/2Pre-PL/7		30.0	32.0		13		0.00	0.00	○
12/1	32			30.0	31.0		13		0.00	0.00	○
12/2	33	PL/10	320	30.0	31.0	6.80	10	7.23	0.00	0.00	○
12/3	34	PL/10	500	29.0	31.0		6		0.00	0.00	○
12/4	35	PL/10	600	29.0	31.0		3		0.00	0.00	○
12/5	36	PL/10	0	29.0	31.0		3		0.00	0.00	○
Minmum				29.00	30.00	6.44	0.67	7.10	0.00	0.00	
Maximum				31.00	32.00	6.87	13.00	7.71	0.50	0.50	
Average				29.89	31.59	6.69	11.86	7.35	0.04	0.01	