

**Effect of two commercial probiotics on growth performance of fresh
water prawn *Macrobrachium rosenbergii* (De Man, 1879)**



A Thesis

By

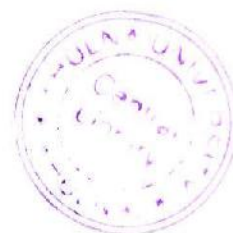
Joyanta Bir

Student No: MS 120602

Session: 2011-2012



This Thesis submitted to Fisheries and Marine Resources Technology Discipline in
partial fulfillment of the requirements for the degree of
MS in Fish Genetics and Biotechnology.



**Fisheries and Marine Resources Technology Discipline
Khulna University
Khulna-9208**

November, 2012

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Approved as to style and content

By

A handwritten signature in black ink, appearing to read "Nazmul Ahsan". The signature is written over a horizontal dotted line.

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Professor Dr. Md. Nazmul Ahsan

Head

Fisheries and Marine Resource Technology Discipline

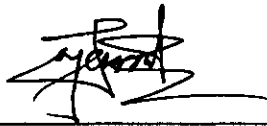
Khulna University

Khulna-9208

Declaration

**Effect of two commercial probiotics on growth performance of fresh water
prawn *Macrobrachium rosenbergii* (De Man, 1879)**

**The results presented in this project thesis with the above mentioned title are entirely of the
candidate's own investigations and no part of the results has been accepted for any degree
nor is it being concurrently submitted for any degree.**



(Signature of Candidate)

Joyanta Bir

Examination Roll No: MS 120602

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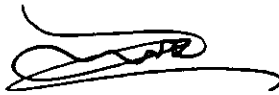


(Signature of Co-Supervisor)

Alokesh Kumar Ghosh

Assistant professor

Fisheries and Marine Resources Technology Discipline
Khulna University, Khulna-9208



(Signature of Supervisor)

Dr. Khandaker Anisul Huq

Professor

Fisheries and Marine Resources Technology Discipline
Khulna University, Khulna-9208

November, 2012

Dedication

**Dedicated to my beloved parents, Grandmother and my sisters who sacrificed for
me, inspired and blessed me in every way**

Acknowledgement

At first all my admiration goes to the Supreme Creator of the universe-God for his great mercy to create and keep me alive to complete this thesis work for the degree of MS in Fish Genetics and Biotechnology from Khulna University under Fisheries and Marine Resource Technology Discipline.

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The author
November, 2012



Abstract

In order to evaluate the production performance of fresh water prawn (*Macrobrachium rosenbergii*) by applying locally available probiotics, a study was carried out in the pond complex II of Fisheries and Marine Resource Technology Discipline, Khulna University from August 2011 to December 2011. There were four treatments named without probiotic (Control T₁), Zymetin (T₂), Super PS (T₃) and Both Zymetin and Super PS (T₄). Twelve ponds with three replicates for each treatment group and the control group were used and the stocking density was 2/m². Initially at the time of stocking average body weight of prawn was 1.04g. After 4.5 months, at the time of harvesting average weight increased to 24.93g in control pond, 30.48g in Zymetin treatment pond, 36.27g in Super PS and 40.06g in combine application of Zymetin and Super PS pond. The production of control pond was 397kg/ha where as 492kg/ha for only Zymetin treated pond, 582kg/ha for only Super PS treated pond and 656kg/ha for both Zymetin and Super PS treated pond. Probiotics treated pond production was always higher than control pond. The mean weight and length of each treatment groups was significantly different ($P < 0.05$) from that of the control. There was no significant difference of water and soil parameters among the treatments. Water pH was 7.76-7.85 while temperature was 25-30 °C in dry period, and 12.5-18.5 °C in winter, salinity 0.5ppt, hardness 380-450mg CaCO₃/L, alkalinity 180-240mg CaCO₃/L, dissolved oxygen 4.5-5.3 ppm, transparency 40-60cm ammonia and nitrite were 0.3-0.8 ppm and 0.2-0.7ppm respectively. The soil pH was 6.7-7.7, while organic carbon was 1.067 to 1.32 % Ca, 0.13 to 0.17%; Mg 0.08-0.09%; phosphate 0.02-2.67%; electrical conductivity (EC), was 2.3to 6.91 ds/m. Maximum microbial load was found in non probiotic treated pond water (5.3×10^5 CFU/g)while minimum in Super PS treated pond (3×10^4 CFU/g).

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List of abbreviations

GDP	Gross Domestic Products
cm	Centimeter
DoF	Department of Fisheries
g	Gram
ppt	Parts per thousand
CFU	Colony forming unit
FMRT	Fisheries and Marine Resource Technology
EC	Electrical conductivity
DO	Dissolved oxygen
TL	Total length

Chapter 1
INTRODUCTION



Chapter 1

Introduction

Bangladesh is a small country of south Asia. It is a deltaic country and enriched with water resource. Bangladesh has extensive areas of fresh water, brackish water and marine water. Fisheries sector plays a significance role in the economic welfare in Bangladesh. In 2010-2011 total fish production was 30.62Mt, which market value was about 4603.83 core taka. Fisheries sector contribute 4.43% of national GDP and 22.21% in agricultural production and 2.73% in export earnings (Anon, 2011-12). Fish supply about 60% animal protein of our meal. About 12.80 million people are engaged in the exploitation and conservation of fish and fisheries products. (Matsya Pakkaha, 2008-2009) Prawn is one of the important commercial arthropod in Bangladesh. Our condition and weather is suitable for prawn culture. At present our shrimp and prawn production rate 669kg/ha (Anon, 2011-12 where Thailand 2338kg/ha (Vicki, 2007), Vietnam 1000-1500 kg/ha (Ridmontri, 2002), China 1500kg/ha (Welmin and Xlanping, 2002), India 600-1000kg/ha (Ralzeda *et. al.*, 2005). The global annual production of freshwater prawns (excluding crayfish and crabs) in 2003 was about 280,000 tons, of which China produced some 180,000 tons, followed by India and Thailand with some 35,000 tons each. In 2010-11 fiscal year Bangladesh produced 239460 Mt. Shrimp and Prawn from the farm. (Anon, 2011-12).

Aquaculture has been increasingly proved as an important source of animal protein for the world's growing population, especially for developing countries where urban and rural areas are getting over-populated. Our annual fish demand 20.44 kg per capita but we intake only 18.94kg per capita (Anon, 2011-2012). To meet the increasing demand for staple diet and high-quality food around the world, aquaculture has globally been expanding into new directions, intensification and diversification. With the increasing intensification and commercialization of aquaculture production, disease problems inevitably emerged. Disease has been a primary constraint to the culture of many aquatic species, impeding both economic and social development in many countries. In recent decades, disease prevention and control have led to a substantial increase in the use of antimicrobial drugs, pesticides, and disinfectants. The utility of antimicrobial agents as a preventive measure has been questioned; the abuse of antimicrobial

drugs, pesticides, and disinfectants in aquaculture disease prevention and growth promotion has led to the evolution of resistant strains of bacteria and questions of safety (Esiobu *et al.*, 2002).

A freshwater prawn farm is an aquaculture business designed to raise and produce freshwater prawn or shrimp for human consumption. With increasing demand for environment friendly aquaculture, the use of prebiotics in aquaculture is now considered as alternatives to antibiotics and chemicals to improve the quality and sustainability of aquaculture production (Li *et al.*, 2006). The use of probiotics in the culture of aquatic organisms is increasing with demand for more environment friendly aquaculture practices. (Gatesoupe, 1999) Probiotic is defined as 'live microorganisms, conferring a healthy benefit on the host when being consumed in adequate amounts' (FAO/WHO., 2001). The term probiotic has been used to include bacteria, or mixtures of bacteria, added directly to the water to influence the microbial environment of the culture system, as well as bacteria added through the feed to colonise the gut and improve feed utilization and animal health through the action of beneficial microorganisms in the gastrointestinal tract of the host (Gatesoupe, 1999).

Bangladesh with its favorable resources and agro-climatic conditions is widely recognized as one of the most suitable countries in the world for giant freshwater prawn (*Macrobrachium rosenbergii*, De Man, 1879) farming. The farming of freshwater prawn is one of the most emerging economic activities in southwest regions of Bangladesh, and over the last two decades, its development has been significantly tangible because of its export potential. The prawn culture area extended from 6,000 ha in 1994 (Chanda and Khondaker, 1994) to an estimated 50,000 ha (Khondaker, 2007).

The use of probiotics as a means of reducing disease is widely accepted on the principle of competitive exclusion of potentially pathogenic microorganisms; digestion enhancement by participating in digestion process, supplying fatty acids and vitamins; and providing necessary growth factors (Balcázar *et al.*, 2006; Vine *et al.*, 2006); immune response enhancement through modulating non specific immune responses; water quality improvement by enhancing the domination of heterotrophic bacteria; and antiviral effects through the production of antagonistic compounds. Intake of probiotics could improve intestinal microbial balance, leading to enhanced

absorption of food, increased activity of digestive enzyme and reduced pathogenic problems in the gastrointestinal tract (Balcazar. 2003)

Although application of probiotics in aquaculture seems to be relatively recent, the interest in such environment friendly treatments is increasing rapidly. Increased immunity and disease resistance of *Penaeus monodon* against *Vibrio harveyi* has been shown following administration of the Bacillus S11 probiont in the feed (Rengpipat *et al.*, 2000). Villamil *et al.* (2002) evaluated that *Lactococcus lactis* caused the major increases in immune functions of turbot. Gatesoup (2008) reported that feeding with Gram-positive as well as Gram negative selected probiotics, caused an increase in cellular parameters such as the number of erythrocytes, lymphocytes and macrophages and enhanced lysozyme activity. This species (as well as other *Macrobrachium*) is commercially important for its value as a food source. (T. Y. Chan, 1998)

M. rosenbergii is considered a freshwater species, the larval stage of the animal depends on brackish water. (F. Wynne, 2000) Once the individual shrimp has grown beyond the planktonic stage and become a juvenile, it will live entirely in freshwater. Males can reach total length of 320 mm; females 250 mm.

Scientific classification of *Macrobrachium rosenbergii*

Kingdom: Animalia

Phylum: Arthropoda

Subphylum: Crustacea

Class: Malacostraca

Order: Decapoda

Infraorder: Caridea

Family: Palaemonidae

Genus: *Macrobrachium*

Species: *Macrobrachium. rosenbergii* (De Man, 1879)

Riverine giant prawn (*Macrobrachium rosenbergii*) farming is currently one of the crux sectors of the national economy in Bangladesh with its diverse contributions such as food production, employment opportunity for income-poor fisher-folks, and valuable foreign currency earnings.

Prawn farming has been expanding rapidly in recent years, an average 10-20% per annum (Khondaker, 2007). The area of prawn farming has been estimated to be 30,000 ha (Alam *et al.*, 2007) extended from 6000 ha in 1994. Prawn farming is well suited to integrate with paddy production and culture of certain fish species. Although freshwater prawn is rather less susceptible to viral diseases than shrimp, this production sector encounters diseases. In 2010-2011 fiscal years the area of prawn and shrimp farming has been estimated to 276492 ha. Anon, 2011-12).

Conventionally farmers use chemicals and antibiotics in their prawn farming, and very recently they have been oriented with the probiotics, and employing them in their hatchery and grow-out ponds considering the demand for more disease-free, environment-friendly and sustainable aquaculture practice. There are increasing reports of research works on the application of probiotics in aquaculture (Hai *et al.*, 2009; Zhou *et al.*, 2009; Li *et al.* 2006). Very few studies related to the usage of probiotics in aquaculture are available in Bangladesh. Sarker *et al.*, 2008 reported that the probiont *Bacillus* sp. isolated from coastal waters of Bay of Bengal could be used in shrimp hatchery and grow-out ponds to control vibriosis.

Super PS is a beneficial microbial culture aimed to improve shrimp growth and pond bottom condition. It is composed mainly on *Rhodobacter* and *Rhodococcus* that is designed to biodegrade soluble organic pollutants of the pond. *Rhodobacter* is a kind of purple bacteria; a group of bacteria that can obtain energy through photosynthesis. Its best growth conditions are anaerobic phototrophy (photoheterotrophic and photoautotrophic) and aerobic chemo heterotrophy in the absence of light (Mackenzie *et al.*, 2007). *Rhodobacter* is also able to fix nitrogen. It is remarkably metabolically diverse, as it is able to grow heterotrophically via fermentation and aerobic and anaerobic respiration.

Rhodococcus is commonly found in dry and dusty soil which is a genus of aerobic, nonsporulating, nonmotile Gram-positive bacteria (Vander, 2004). *Rhodococcus* are applicably important owing to their ability to catabolize a wide range of compounds and produce bioactive steroids, acrylamide, and acrylic acid, and their involvement in fossil fuel biodesulfurization. *Rhodococcus* have the ability to metabolize harmful environmental pollutants, such as toluene,

naphthalene, herbicides, and PCBs from soil and water. Super Photosynthetic Bacteria is a Phototroph bacteria composition which is effective to solve abundance of organic matter problems at the bottom of shrimp ponds.

Zymetin produced from beneficial bacteria to balance the gut flora in shrimp that aim to improve the health of the shrimp. Control pathogen bacteria in the gut system by produce inhibitor substance and enhance the immune systems in shrimp. Increase the digestibility by enzyme amylase, protease and lipase etc produced by beneficial bacteria. Get better growth rate and good FCR. Assure the good crop by reducing the risk. The beneficial bacteria can reduce the pollution by decompose & utilize feed left over and waste to non toxic form. It is usually mixed with feed to inhibit the growth of pathogenic bacteria in the gut. It also improves the appetite of the shrimp to increase growth rate and reduce FCR. (C P aquaculture (India) private limited)

Control of water quality is the key factor for a successful culture of aquatic animals like fish, prawn and shrimp. An open water system with sufficient good water quality may ensure successful aquaculture. However, if proper quality or sufficient quantity of water is not available, purification of water by eliminating polluting substances including toxic metabolites and growth inhibiting substances which originate mainly from fish or shrimp excretion and excessive feed is necessary. Under these lower water quality conditions there are chances for occurrence of mortalities by diseases due to viral, bacterial, protozoal, fungal pathogens. High populations of aquatic animals can be kept healthy under successful semi-closed and closed systems by maintaining proper sanitisation. The increased demand in water quality and quantity in shrimp and fish culture has resulted in a growing interest in using water sanitisers and disinfectants. Proper pond and water quality management is essential for successful and quality shrimp production. Maintaining a good culture environment through proper management practices can reduce the risk of disease and increase production and acceptability of shrimp. Therefore, it is essential to implement and follow good aquaculture management practices to ensure shrimp quality.

Water quality is the key factor for a successful culture of aquatic animals like fish, prawn and shrimp. An open water system with sufficient good water quality may ensure successful

aquaculture. However, if proper quality or sufficient quantity of water is not available, purification of water by eliminating polluting substances including toxic metabolites and growth inhibiting substances which originate mainly from fish or shrimp excretion and excessive feed is necessary. Soil nutrition is only one of more than fifty factors which directly affect both yield and quality of water. The availability of required nutrients, together with the degree of interaction between these nutrients and the soil, play a vital role in water quality development. A deficiency in any one required nutrient or, a soil condition that limits or prevents a metabolic function from occurring can limit the growth. A soil nutrient management plan should include analyzing soil deficiencies to determine the type, application rate, application interval, and the placement of any nutrients required to optimize short and long term productivity. There is a significant difference between an induced deficiency and a real soil deficiency.

Soil nutrient management involves not only the physical properties and mineral structure of the soil, but also the balance between soil pathogens and beneficial microbes. Beneficial microbes increase nutrient availability, reduce disease, reduce nutrient losses, and help degrade toxic compounds. Organisms thrive or suffer, depending on the type of microbes in the soil. If soil is dominated by beneficial microbes, organism life then soil life work together to produce healthy production. Conversely, in unhealthy soil, dominated by pathogenic microbes, optimum growth is unattainable.

The study work has been developed considering issues such as eco-friendly aquaculture practice, foreign currency earnings through export, and potential prospects of aqua-vet market for probiotics. The results from the study will help the prawn-farmers and aqua-researchers practice sustainable prawn farming. The outcomes from the research will promote probiotics in sustainable prawn culture. The scope of using commercially probiotics in grow-out phase and hatchery phase of prawn culture industry might be developed, and expanded. The success of the study will pave the way for sustainable use of probiotic which requires continuous production and un-interrupted marketing of probiotics within Bangladesh.

Objectives of the study

- To assess the effectiveness of commercial probiotics available in Bangladesh to increase prawn production
- To evaluate the level of prawn production between with probiotics and without probiotics.
- To determine the impact of probiotics on water and soil quality

Chapter 2
REVIEW OF LITERATURE

Chapter 2

Literature review

It is very important to review the past research works which are related to the proposed study. The objective of this chapter is to review the past research works which are relevant to the present study.

In Bangladesh, a few studies have so far been made to evaluate the growth, survival and production of *P. monodon* under the semi- intensive probiotic framing with formulated feed in high stocking density. Hossain (1987) observed some physio- chemical parameters of tide- feed shrimp ponds. Mahamud *et al.* (1993) studied the limnology of the experimental shrimp ponds in Chandpur. Balcazar *et al.* (2006) reported antiviral activity from *Pseudomonas* sp., *Vibrios* sp., *Aeromonas* sp., obtained from salmon hatcheries against infectious hematopoietic necrosis virus (IHNV) with more than 50% plaque reduction.

Soundarapandian *et al.* (2008) studied that applied probiotic in culture ponds of *P. monodon* and was compared with control ponds (without probiotic) the Average Body Weight (ABW), the total production and survival were higher in probiotic applied ponds and Feed Conversion Ratio (FCR) were lower in probiotic ponds. The shrimp of control ponds were seriously affected with various viral diseases.

Bangladesh is considered one of the most suitable countries in the world for giant freshwater prawn (*Macrobrachium rosenbergii* De Man 1879) farming, because of its favorable resources and agro-climatic conditions. A sub-tropical climate and a vast area of water bodies provide a unique opportunity for the production of *Macrobrachium spp.* Twenty-four species of freshwater prawns including 10 species of *Macrobrachium* are found in Bangladesh. However, only *M. rosenbergii* has significant aquaculture potential and is commercially cultured (Akand & Hasan 1992; Ahmed 2001; Muir 2003a).

It has been reported that use of *Bacillus* sp., the major percentage of Zymetin improved water quality, survival and growth rates and the health status of juvenile *Penaeus monodon* and reduced the pathogenic vibrios (Dalmin *et al.*, 2001).

Wang *et al.* (2005) reported that the use of commercial probiotics in *Penaeus vannamei* ponds can reduce concentrations of nitrogen and phosphorus and increase the shrimp yields. *P. synxantha* and *P. aeruginosa* are potential probiotics for use in the culture of western king prawns (Hai *et al.*, 2009).

The use of probiotics in the culture of aquatic organisms is increasing with the demand for more environment-friendly aquaculture practices (Gatesoupe, 1999). Probiotics is the use of microbial supplements to benefit their host (Fuller, 1989). Although application of probiotics in aquaculture seems to be relatively recent (Kozasa, 1986), the interest in such environment friendly treatments is increasing rapidly.

Rengpipat *et al.* (1998a) experimented that Probiotic feeds were prepared by mixing fresh *Bacillus* S11 with commercial shrimp feed pellets at a ratio of *Bacillus* to shrimp feed = 1:3 (wet weight). The mixture were oven-dried at 37 °C for 1–2 h before storing in sealed plastic bags at -20 °C until used. Final concentration of *Bacillus* S11 in treatment feeds was determined to be approximately 10¹⁰ CFU/g feed.

Moriarty (1998) proposed to extend the definition of probiotics in aquaculture to microbial “water additives.” A growing number of studies have dealt explicitly with probiotics, and it is now possible to survey its state of the art, from the empirical use to the scientific approach (Mohanty *et al.*, 1996; Gatesoupe, 1999; Gomez-Gil *et al.*, 2000; Sharma and Bhukhar, 2000; Wang *et al.*, 2005; Wang and Xu, 2006; Vine *et al.*, 2006; Wang, 2007; Kesarcodi-Watson *et al.*, 2008).

Ding *et al.*, (2004); and Ziaei-Nejad *et al.* (2006) studied that the exogenous enzymes produced by the probiotic would represent, at most, only a small contribution to the total enzyme activity of the gut and the presence of the probiotic might stimulate the production of endogenous enzymes by the shrimp larvae. The observed increases in specific activities of digestive enzymes in probiotic treatments might have led to enhanced digestion and increased absorption of food, which in turn contributed to the improved survival in *P. vannamei*.



The potential benefits of probiotics in aquaculture ponds include improvement of water quality, enhancement of nutrition of host species through the production of supplemental digestive enzymes, lower incidence of diseases and greater survival, and improved immune response (Boyd and Massaaut, 1999; Verschuere *et al.*, 2000). Although probiotics for human and terrestrial animals are dominantly lactic acid bacteria (LAB), many different genera, including photosynthetic bacteria, yeast, *Bacillus* and *Lactobacillus* have been evaluated as probiotics in fish and shellfish (Gatesoupe, 1999; Vine *et al.*, 2006; Kesarcodi-Watson *et al.*, 2008). However, few studies have been carried out on spore forming lactic acid producing bacteria such as *Bacillus coagulans* (*B. coagulans*) as probiotics in larval shrimp.

Since the first use of probiotics in aquaculture, a growing number of studies have demonstrated their ability to control potential pathogens and to increase the growth rates and welfare of farmed aquatic animals (Gatesoupe, 1991; Lara-Flores *et al.*, 2003; Carnevali *et al.*, 2004; Macey and Coyne, 2005; Wang *et al.*, 2005; Wang and Xu, 2006)

Allan *et al.* (1995) have also stated that the bacterial counts and bacterial productivity were two orders of magnitude greater in the sediments than in the water columns. Similarly, Smith (1998) observed that *Vibrio spp.* were predominant in the sediment during the farming phase than in the unfarmed sediment samples. Moriarty (1998) reported that vibrios are fermenters and grow well anaerobically at the bottom of the pond.

Bacterial probiotics could answer the requirement of controlling potential pathogens and protecting fish health, preventing them from disease (Gatesoupe, 1989, 1991; Maeda and Liao, 1992; Nogami and Maeda, 1992; Strøm and Ringø, 1993; Carnevali *et al.*, 2004). Some of the numerous disease that can rise inside the rearing tanks are favoured by a stress state of the reared animals due to high stocking densities, handling and poor water quality.

Probiotics is a traditional idea in the field of human dietetics (which accounts for the consumption of fermented lactic products) and is extended to animals by promoting the development of fortifying diets for the intestinal microbiota, which improve feeding yields and

survival. Probiotic development in aquaculture began after the discovery that bacteria in the intestinal tracts of healthy animals, or bacteria from the same marine medium, exercised antagonistic effects on pathogenic bacteria (Skjermo and Vadstein, 1999; Makridis *et al.*, 2000; Hjelm *et al.*, 2004).

The most rapid and experimentally simplest step for the testing of potential probiotic effects consists of determining the inhibitory activity of the cell-free media of different probiotics on pathogenic bacteria associated with larval cultures. In spite of the schematic approach, the study of such activities in several characteristic cases has demonstrated a series of complex profiles (Cabo *et al.*, 1999; Murado *et al.*, 2002; Planas *et al.*, 2003), not describable with conventional dose-response models (Murado *et al.*, 2002).

Ponds sediment plays an important role in nutrients cycling by retaining or releasing nutrients. Moriarty (1996) strongly advocated the use of probiotics amendments in aquacultural pond. Suhendra *et al.* (1997) found that routine use of commercial probiotics in a shrimp farm in West Java resulted in reduced organic matter accumulation, improved water quality and enhanced environmental conditions.

Probiotic development in aquaculture began after the discovery that bacteria in the intestinal tracts of healthy animals, or bacteria from the same marine medium, exercised antagonistic effects on pathogenic bacteria (Skjermo and Vadstein, 1999; Makridis *et al.*, 2000; Hjelm *et al.*, 2004).

Avnimelech *et al.* (1995) explained during intensive shrimp culture process, it was common to accumulate high density of organic material in the pond bottom originated from unused feed, feces and plankton die-offs. As a consequence, nutrient (N, P and C) level in pond sediment are usually higher in the final phase compared with the starting phase through the accumulation. These findings were similar to that of Green and Boyd (1995), who reported that significantly greater N, P and organic matter concentrations were in pre-drain samples, indicating pond sediment was a major nutrient sink.

Shrimp culture disease control using nonpathogenic probiotic bacteria for disease prevention has received wide spread attention during the past decade. Gram *et al.* (1999) reported a broader range of live microbial supplement (probiotics) which beneficially affects the host animal by improving its microbial balance.

Similarly, Moriarty (1998) and Rengpipat *et al.* (1998) have reported an increase in shrimp survival and a decrease of pathogenic luminous *Vibrio spp.* in the sediments and water in the ponds where some strains of *Bacillus spp.* were introduced. Vaseeharan and Ramasamy (2003) reported that pathogenic vibrios were controlled by a probiotic strain *Bacillus subtilis* BT23, under laboratory conditions.

In intensive aquaculture, growth and proliferation of microorganisms in the rearing habitat are accelerated by excessive feeding (Olafsen, 2001). Successful aquaculture relies on adequate microbial control to prevent the proliferation of pathogenic bacteria that often results in fish mortality. Bacteria can accumulate in fish by ingestion or through the body surfaces and is transported by the body fluids into the tissues. This may be one of the causes of indigenous micro flora formation and bacterial infection in fish.

The decrease of pH in the water supply group was distinct compared to the control and the probiotic diet groups. The difference may be due to acid production by bacteria, because commercial probiotics used in this study included acid-producing bacteria. Rengpipat *et al.*, 1988 reported that probiotics (*Bacillus*) added to shrimp diets did not affect water quality. However, when *Bacillus* was added to the rearing water, the water quality slightly improved.

Nageswara and Babu, (2006) reported that in the process of application probiotics, no other chemical or drug should be used for testing other diseases. These probiotic can easily be destroyed by any other chemical or drug which generally interferes with the establishment of useful microbes. When the probiotics treatment is applied the probiotics will suppress the growth of pathogenic bacteria by the competitive exclusion principle (Sayani, 1995).

Havenaar *et al.* (1992) studied that the use of beneficial bacteria (probiotics) to displace pathogens by competitive processes is being used in the animal industry as a better remedy than administering antibiotics and is now gaining acceptance for the control of pathogens in aquaculture.

Smith, 1993 studied that Luminous *Vibrio* were completely eliminated from the water column and from the sediment of ponds in Indonesia when probiotic strains selected for their direct inhibitory effect were used. In contrast, *Vibrio* numbers increased markedly from around 20 to over 200 cfu/ml in shrimp ponds where antibiotics were used in the feed. Survival, and thus production, was high in all ponds where probiotics were used.

CHAPTER 3
MATERIALS AND METHODS

Chapter 3

Materials and Methods

3.1 Materials

3.1.1 Baseline survey

A baseline survey was conducted in Dumuria, Rupsha, Batiaghata and Fultala under Khulna District and Fakirhat and Rampal upazilla under Bagerhat District; and Jessore, Narail and Gopalganj Districts to know the existing prawn culture practiced (pond preparation, water and soil quality management, types of food, stocking density, PL nursing, disease control and health management and overall prawn production etc).

3.1.2 Experimental Design

The study was conducted for six months. But only 4.5 months belonged for culture period another 1.5 months used for rearing larvae in hatchery. In this time the impact of the selected two probiotics on the production performance of prawn at grow-out phase was analysed. The study was conducted for four treatments with three replications. The four treatments were as 'without probiotic', 'only Super PS', 'only Zymetin', and 'Super PS + Zymetin'.

3.1.3 Experimental ponds

The experiment was conducted in pond complex II of Khulna University campus. There were five small ponds and seven large ponds in the experimental site. The size of each large ponds was 250 m² and small pond size was 110m². The ponds were rectangular in shape and were numbered.

Table 1. Name of treatment in different culture ponds

Treatment	Description of Treatment	Mode of Application and Doses	Stocking Density	Used Feed	Remarks
T1 Pond no: 1,5, 11	Control	Without Probiotics	2/m ²	Mega starter feed (40% protein) during nursing in	1.Each treatments was eplicated

T2 Pond no 2, 6, 8	Zymetin	5g Zymetin witt 20 g Mutagen binder/kg feed/four days in a week	2/m ²	first two months and Mega grower (32% protein) for the rest 4.5 months	three times 2. During nursing stocking density was 20PL/m ²
T3 Pond no 3, 7, 9	Super PS	15 Litre with 7.5 kg Sand /ha/week	2/m ²		
T4 Pond no 4, 10, 12	Super PS + Zymetin	Both above dose	2/m ²		

3.1.4 Pond preparation

Pond preparation is the essential requirement before stocking of fish. Better condition of pond is essential for the better growth as well as for the survival of fry. To ensure the adequate primary production in the ponds before stocking, pond preparation was done three weeks before stocking of fry. Steps of pond preparation were as follows:

3.1.4.1 Dewatering, drying and digging

Initially the pond was with water and black mud on the bottom and was not suitable to conduct research. The pond was first dewatered with pump and dried under sun light for one month to excavate the bottom mud and to done digging of the experimental ponds; thus automatically aquatic weed and unexpected fauna were removed. The embankment of the pond was repaired.

3.1.4.2 Liming and Watering

Liming was done after 2-3 days of drying. Liming controls the pH that balances acidity and alkalinity of soil and water. Liming dose depends upon soil conditions of the ponds. Calcium carbonate (CaCO₃) and dolomite was used as a liming agent. After testing pH liming was done at a dose of 1kg/decimal that helps to maintain good water quality. Watering was done after liming. The ponds ware filled up with fresh water up to a depth of about 1.5 meter. Ground water was used in culture pond. The water level is good now due to the continuous heavy rain.

3.1.4.3 Fencing, Predator control and observation of natural feed

The total area of the ponds was fenced with nylon net so that undesirable organisms cannot enter into the pond from outside. Bamboo pole was used to enhance the strength of the fence. In order to control predator and other undesirable fishes, amphibians, reptiles, insects, frogs' etc, bleaching powder dose was used. It is also used for controlling germ and harmful microbes. Bushes on the banks of the ponds were removed manually to destroy the habitat of other predators like otters, snakes, etc. Within 3-4 days after fertilization the water of the experimental ponds turned green color indicating that adequate plankton had been produced.

3.2 Methods

3.2.1 PL Nursing and stocking

After pond preparation, prawn post larvae nursing was done in the pond to reach the PL into juvenile to reduce the mortality rate of prawn into grow out pond. The PL was nursed for 45 days from June 15 to July 30, 2011. After nursing the PL for 45 days, PL were stocked in grow out pond on 1st August when the average individual weight was 1.02g. Before releasing in the pond, acclimatization of fry in the new environment was done by keeping the pots in pond water for a while and then gradually mixing pond's water with fry containing water, which was very important to reduce mortality. After stocking, prawn feed (35% protein level) at 10% of their body weight was used at the first time. The water quality was maintained in the acceptance level. Two probiotics namely Super PS and Zymetin are being used according to the label of instruction into the experimental pond to know the effects of these which is the ultimate target of this study.

3.2.2 Probiotic treatment

Zymetin was treated first four day in a week, but Super PS was treated every Friday in a week. Super PS was applied in a dose of 15 L/ha at 9 am to 11 am every Friday. Before application, super PS was mixed with sand (Dose 3kg sand/6L super PS) and if pH below 7 then super PS mixed spread it through the feeding line. The dose of Zymetin was 5g/kg feed in one meal per day. Before using, Zymetin were mixed with Mutagen and finally those mixed with food and given first four days in a week.

3.2.3 Sampling and Growth measurement

Sampling was done regularly at fifteen days interval. An average 50 Samples were collected from each pond by using cast net. Then total length and body weight were measured. Total length of prawn sample was recorded in centimeter (cm) from the tip of carapace to telson. Body weight was recorded by electric balance of 0.001 g accuracy. First we caught the sample and kept into the Hapa. After collecting all samples we measured length through a scale and weight by Electric balance.

3.2.4 Measurement of Water quality

Different parameters of the water quality were measured in the water chemistry laboratory.

3.2.4.1 Measurement of Salinity, pH and Temperature

Salinity was measured by a hand Refractometer. pH was measured by pH meter and thermometer was used to measure temperature. Salinity was recorded at 15 days interval while pH and temperature were recorded regularly.

3.2.4.2 Measurement of Hardness and Alkalinity

Hardness is measured by the following equation

$$\text{Hardness (EDTA) as mg CaCO}_3/\text{L} = \frac{A \times B \times 1000}{\text{ml sample}}$$

Here, A=Amount of titrate

B= Normality (0.1N)

Alkalinity is measured by the following equation

$$\text{Alkalinity, CaCO}_3 \text{ mg/L} = \frac{A \times N \times 5000}{\text{ml sample}}$$

Here, ml sample

A= mean value

N=Strength of H₂SO₄

3.2.4.3 Measurement of Nitrite and Ammonia and Dissolved oxygen.

Ammonia and Nitrite were measured by using kits and dissolved oxygen was measured by DO meter.

3.2.5 Length weight relationship of sample

Length- weight relationship was calculated by using LeCren's formula, as follows

$$W = aL^b,$$

Where W= weight in gram,

L= length in cm,

a= intercept and

b= exponent

The exponential form of relationship in the formula can be expressed in the logarithmic form

$$\log W = \log a + b \log L$$

Value of a and b calculated using following formula by Rounsefel and Everhart (1953), Lagler (1956) and Dewan and Doha (1979)

$$\log a = \frac{\sum (\log L)^2 - \sum \log L \times \sum (\log L \times \log W)}{N \times \sum (\log L)^2 - (\sum \log L)^2}$$

$$\text{And } b = \frac{\sum \log W - N \times \log a}{\sum \log L}$$

Where, N= Sample size
W= weight in gram (g) and
L= Length in cm

The regression co-efficient b (slope of regression line of weight to length) was calculated using LeCren's (1951) formula.

$$r = \frac{\sum xy - \frac{\sum x \cdot \sum y}{N}}{\sqrt{\left\{ \sum x^2 - \frac{(\sum x)^2}{N} \right\} \left\{ \sum y^2 - \frac{(\sum y)^2}{N} \right\}}}$$

3.2.6 Microbial analyses

Total plate count of bacteria was done by tryptic glucose yeast Agar medium. The laboratory tests were done in the Microbiology Lab of Fisheries and Marine Resource Technology Discipline, Khulna University. About 10 prawn samples used to calculate the result.

3.2.6.1 Standard plate count

Culture media preparation

Plate Count Agar (PCA)

3.525 g of plate agar was first added to 150 ml of distilled water and boiled to dissolve the medium completely. The medium then sterilized by autoclaving at 15 ibs pressure and 121 °C for 15 minutes

Procedure

20g of pooled sample was added to 180ml of 0.1% peptone water and blended for 2 minutes

Ten fold serial dilutions were made (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} , and 10^{-5}) in the McCarty's bottle

1.0ml of each dilution is transferred into sterile Petri dishes

20.0ml of autoclaved and molten Tar agar was given into each Petri dishes

Petridishes are incubated at 37°C for 48 hours

Plate within 30-300 colonies were counted

Calculation was done as cfu/g

3.2.7 Statistical analysis

Relationship between total length and treatment, body weight and treatment and total length and body weight were estimated by using a SPSS 16 and Microsoft excel.

CHAPTER 4

RESULTS

Chapter 4

Results

4.1 Measurement of Total length of Prawn

Length of prawn increased in each sampling. At the time of stocking length of prawn was 2 to 3cm. After 15 days of stocking, 1st sampling was done when the average length was near about 6.38cm in control pond, 6.8cm in Zymetin treatment pond, 6.26cm in Super PS and 6.06cm in combine application of Zymetin and Super PS pond. But at time of 9th sampling the average length of prawn of control pond was 12.91cm. Whereas the average length of Zymetin, super PS and combine application of Zymetin and Super PS treatment pond was 13.99cm, 14.28cm and 14.58cm respectively (Figure 1). It indicate that there was significant different among the treatment. Mean total length of the samples was found to vary significantly ($P = 0.03 < 0.05$) while One Way ANOVA was performed (Table 1). And Pearson correlation length of the sample was significant at 0.01 labels (2 tailed) Table 3.

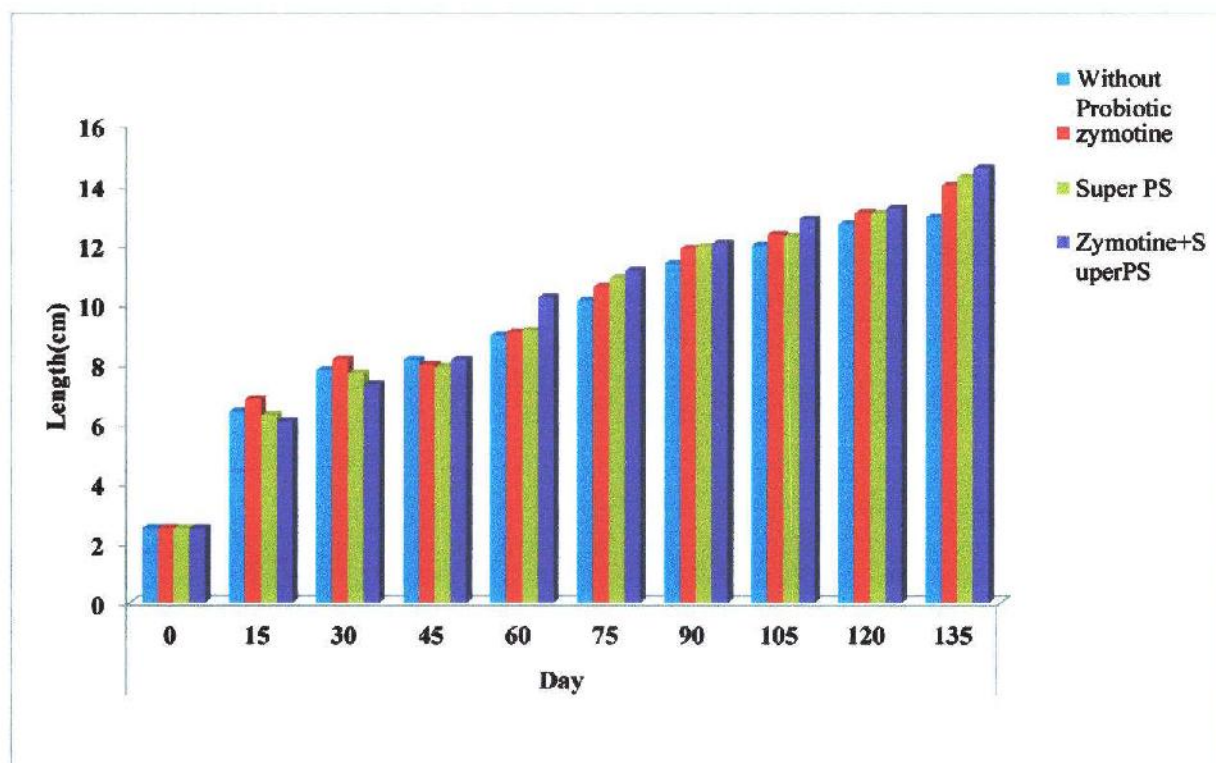


Figure1: Average Length (cm) in four treatments from August to December, 2011

Table 1. ANOVA table for length

ANOVA					
Length					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	321.940	3	107.313	4.710	.003
Within Groups	127632.559	5602	22.783		
Total	127954.499	5605			

4.2 Measurement of total body weight

Initially at the time of stocking average body weight was near about 1.04g. After 15 days of stocking 1st sampling was done when the average body weight was near about 3.26g in control pond, 3.67g in Zymetin treatment pond, 2.92g in Super PS and 2.86g in combine application of Zymetin and Super PS pond. But at the time of 9th sampling their weight increases to 24.93g in control pond, 30.48g in Zymetin treatment pond, 36.27g in Super PS and 40.06g in combine application of Zymetin and Super PS pond. Mean total weight of the samples was found to vary significantly ($P = 0.00 < 0.05$) while One Way ANOVA was performed (Table 2). And Pearson correlation weight of the sample was significant at 0.01 labels (2 tailed) Table 3. The average growth of prawn is shown by the graph According to the graph (Figure 2) it is shown that the average weight of super PS and mixed treatment shows the highest result. Generally fish growth well in Zymetin treated pond but due to blooming average body weight reduced.

Table 2: ANOVA result for Weight

ANOVA					
Weight					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4731.581	3	1577.194	11.488	.000
Within Groups	769086.182	5602	137.288		
Total	773817.763	5605			

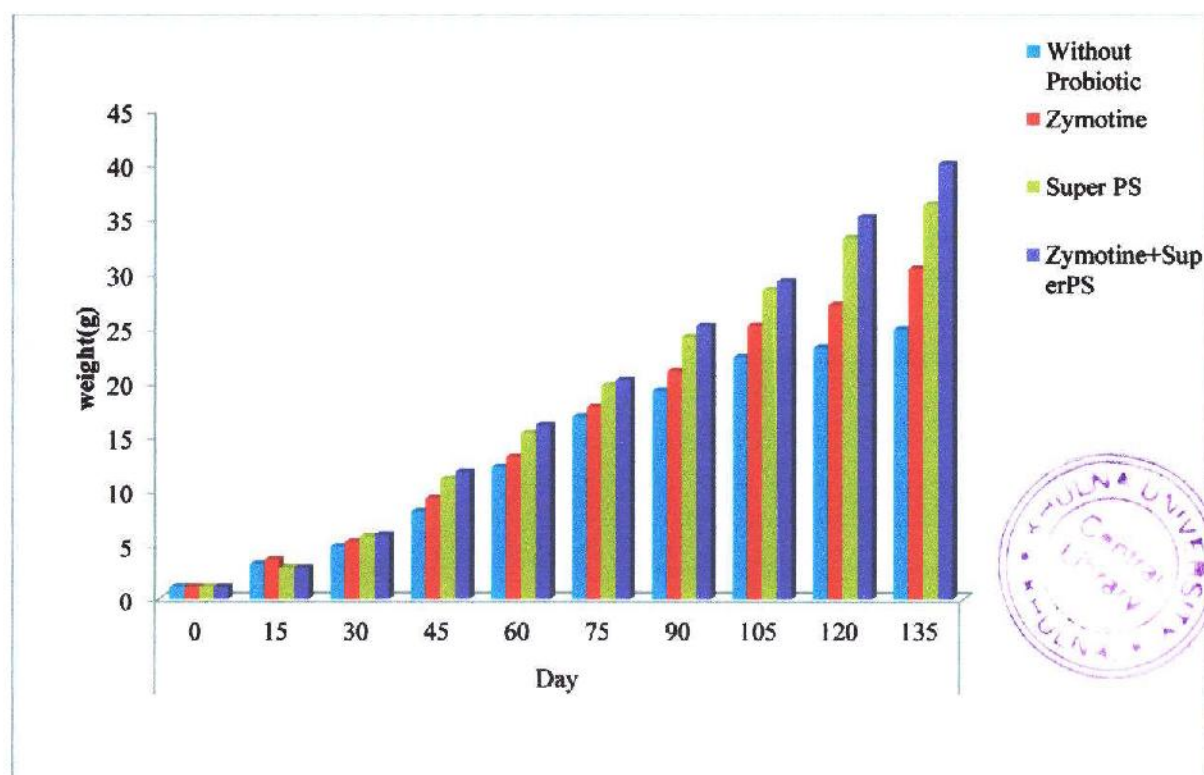


Figure 2. Average prawn weight (g) in four treatments from August to December, 2011.

Table 3. Correlation is significant at the 0.01 level (2-tailed).

Correlations				
		Treatment	Length	Weight
Treatment	Pearson Correlation	1	.044**	.077**
	Sig. (2-tailed)		.001	.000
	N	5606	5606	5606
Length	Pearson Correlation	.044**	1	.479**
	Sig. (2-tailed)	.001		.000
	N	5606	5607	5607
Weight	Pearson Correlation	.077**	.479**	1
	Sig. (2-tailed)	.000	.000	
	N	5606	5607	5607
**. Correlation is significant at the 0.01 level (2-tailed).				

4.3 Length weight relationship of sample

The length weight relationship was established using the formula $W = aL^b$ which, in logarithmic form was $\text{Log}W = \text{Log} a + b \text{Log} L$. The relationship of total length and weight was linear and positive. (Figure 3).

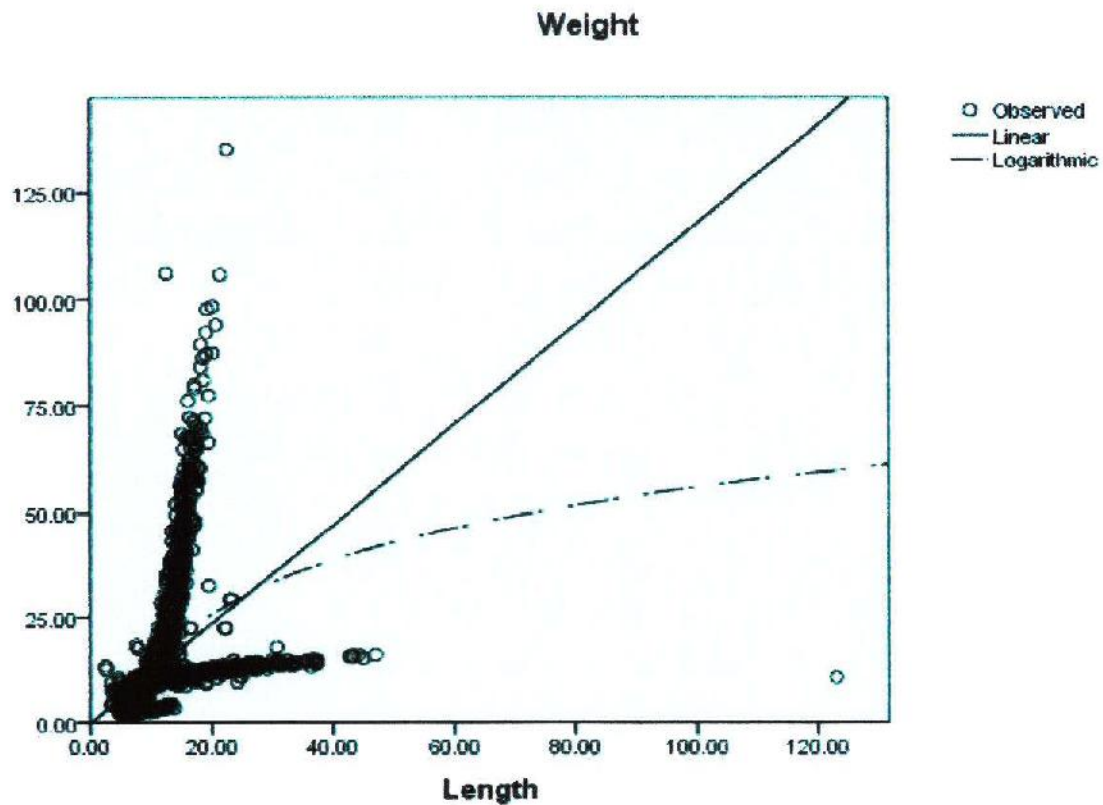


Figure 3. Length weight relationship of prawn sample

4.4 Production performances of prawn

The production period was only four and half months. The total production of without probiotic treated pond was 397kg/ha, where as 492kg/ha for only Zymetin treated pond, 582kg/ha for only Super PS treated pond and 656kg/ha for both Zymetin and Super PS treated pond. So it is found that there was significant different between probiotic treated pond and non probiotic treated pond. Highest production found in both Zymetin and Super PS treated pond about 656kg/ha which is 259kg/ha more than non probiotic treated pond. If the market value of prawn is 500tk/kg, then the of market price non probiotic treated pond is 198500 tk/ha while market price of both Zymetin and Super PS treated pond is 328000tk/ha. The marker price of additional 259kg prawn is 129500tk. If the price of probiotic is 40000tk/ha, then net profit will be 89500tk more than non probiotic treated pond (Table 4).

4.5 Water parameters

In Probiotic culture system water quality was maintained properly. To maintain water quality stability of pH is very necessary. For this regular checking of pH was done , if it was below the optimum level then applied Dolomite (20 kg/acre), if it was higher than optimum level then applied Molasses, Rice bran and Yeast (10 Kg : 5 Kg : 0.1 Kg per acre) , before application it must be fermented by soaking water in previous day. To improve alkalinity agricultural lime was applied in 8-10 Kg/acre.

Water Quality Parameters in Different ponds

4.5.1 pH

pH varied slightly in different ponds in different times. This may varied due to different causes, such as food, water temperature, rainfall, plankton blooming and some other activities. There was not enough different in pH among the treatment. pH was relatively higher (7.85) in Zymetin and Super PS treated pond (Table 5) and lower (7.76) in combine application of probiotic treated pond.

Table 5 .The average pH values of different treatment from August to December, 2011

Name of treatment	Average pH value (ppt)
Without probiotics (P1, P5, P11)	7.8
Zymetin (P2, P6, P8)	7.85
Super PS (P3, P7, P9)	7.85
Zymetin + Super PS (P4, P10, P12)	7.76

4.5.2 Salinity

Salinity indicates how much ion present in water. In this experiment ground water was used. And the water salinity was almost similar among the pond. There is little or no salinity in the water. Salinity ranges from 0-0.5 ppt. this salinity ranges is suitable for prawn culture.

4.5.3 Hardness and Alkalinity

There is no wide difference in hardness and alkalinity among the pond. Generally Hardness varies between 300-450 CaCO_3/L (Table 6) and Alkalinity varies between 180-240 CaCO_3/L .

Table 6. Average hardness and alkalinity of water in different treatments from August to December, 2011

Type of treatment	Hardness (mgCaCO_3/L)	Alkalinity ($\text{mg CaCO}_3/\text{L}$)
Without probiotics	330-450	180-220
Zymetin	300-400	190-220
Super PS	320-430	200-240
Super PS & Zymetin	350-450	180-230

4.5.4 Temperature and Dissolved O_2

These two factors are very important in aquaculture. This change occurred regularly even different period of a day. We measured temperature at three times per day. There was a little bit variation between the times in a day. At the time of study first three months belong to dry season another two months belong to winter. So there was a fluctuation of temperature between two seasons. Generally in dry season the temperature in morning ranges $23-26^\circ\text{C}$ at noon $25-30^\circ\text{C}$

and evening 25-30°C (Table: 7.1 and 7.2). At that time the temperature in morning ranges 12.5-16.5°C at noon 11.5-17.5 °C and evening 11.5-17.5°C.

Dissolved O₂ ranged in 4.5-5.3ppm. and in winter season water temperature when was relatively low DO was also relatively low.

Table 7.1. Average temperature in dry season from August to October 2011

Treatments	Temperature (°C)			DO (ppm)
	Morning	Noon	Evening	
Without probiotics	23-25	25.5-26.5	25.5-30	4.8-5.1
Zymetin	23.5-25.5	26.5-28.5	26.5-28.5	4.6-5.2
Super PS	24-24.5	26.5-28.5	26.5-29.5	4.6-4.9
Super PS & Zymetin	23.5-24.5	25.5-28.5	28.5-29.5	4.8-5.0

Table 7.2. Average temperature and dissolved oxygen from November to December, 2011

Treatments	Temperature (°C)		
	Morning	Noon	Evening
Without probiotics	12.5-15.2	11.5-17.5	11.5-15.5
Zymetin	12.5-15.5	11.5-17	11.5-15.5
Super PS	12.5-15.2	11.5-17.5	11.5-15.5
Super PS & Zymetin	13.5-16.5	12.5-18.5	12.5-17.5

4.5.5 Ammonia and Nitrite

As a result different deformities found in cultivable species. In our culture pond ammonia and nitrite ranged from 0.3-0.8 and 0.2-0.7. In this experiment ammonia and nitrite kits were used to determined ammonia and nitrite. Ammonia and nitrite concentrate in different pond was given in (Table 8). It was found that the concentration of ammonia was high in control and low in Zymetin treated pond, on the other hand Nitrite was high in Zymetin treated pond and lower in mixed pond.

Table 8. Average Ammonia and Nitrite in different treatment from August to December, 2011

Type of treatment	Ammonia (ppm)	Nitrite (ppm)
Without probiotics	0.4-0.8	0.2-0.7
Zymetin	0.4-0.7	0.4-0.6
Super PS	0.3-0.7	0.3-0.7
Super PS & Zymetin	0.3-0.5	0.2-0.5

4.5.6 Water transparency

Average water transparency of the pond was ranged from 40cm to 60 cm. Generally Water transparency ranges 35-45cm is good for closed monoculture system.

4.6. Soil quality test

4.6.1 Soil pH

Soil pH was determined electrochemically with the help of glass electrode pH meter as suggested by Jackson (1973). In this study soil pH was varied from 6.7 to 7.7 (Table 9). The ratio of soil to water was 1:2.5 as suggested by Jackson (1962).

Table 9. Range of soil pH in different treatment.

Treatment name	pH
Without Probiotics	6.9-7.7
Zymetin	6.7-7.6
Super PS	6.7-7.6
Zymetin and Super PS	7-7.6

4.6.2 Determination of organic matter

4.6.2.1 Organic carbon

Organic carbon of soil samples was determined by Walkley and Black's wet oxidation method as outlined by Jackson (1973). Organic matter was calculated by multiplying the percent value of organic carbon with the conventional Van-Bemmelene's factor of 1.724 (Piper, 1950). In this study organic carbon ranged from 1.067 to 1.32 % (Table 10)

Maximum value was found in control pond where minimum in combine application pond (Table 13)

Table 13. Level of Phosphorus in different treatments.

Treatments	P(meq/100g)
Without Probiotics	2.67%
Zymetin	2.33%
Super PS	0.03
Zymetin and Super PS	0.023333

4.6.3 Electrical conductivity (EC)

The electrical conductivity of the soil was measured at a soil: water ratio of 1:2.5 with the help of EC meter (USDA, 2004). In this study electrical conductivity (EC) ranged from 2.3 to 6.91 ds/m. (Table 14). Maximum electrical conductivity found in mixed applicable pond and minimum in control pond

Table 14. Range of electrical conductivity (EC) in different treatments.

Treatments	Ec(ds/m)
Without Probiotics	2.3-6.84
Zymetin	5.2-6.15
Super PS	5.7-6.46
Zymetin and Super PS	5-6.91

4.7 Microbial Analysis

In this study only total microbial load was measured. The microbial load varied from 3×10^4 to 5.3×10^5 CFU/g (Table 15). Maximum found in control pond while minimum in Super PS treated pond.

Table 15. Average bacterial load of prawn sample in different treatments

Treatments	Bacterial load in prawn meat (CFU/g)
	Average Bacterial load (CFU/g)
Without Probiotics	5.3×10^5
Zymetin	5.3×10^5
Super PS	3×10^4
Super PS+ Zymetin	7.7×10^4

CHAPTER 5
DISCUSSION

Chapter 5

Discussion

In the present study length and weight was measured two times in a month. Length increased in each sampling. From the result it was found that the average length of Super PS and mixed treatment were higher. The value of total length when plotted against corresponding weight, the regression line drawn showed a linear relationship. This relationship indicated that weight of prawn increased with length. This length weight relationship of prawn agreed well with the results reported for *Macrobrachium rosenbergii* (Wang, J. K., 1985), (Cohen, *et.al.*, 1981).

A study of length weight relationship in *Penaeus indicus* was made by Hall (1962) for the singapur area and Brusher (1972) for the Indian waters. Hall showed linear relationship between carapace length and weight without dividing sexes, and Brusher (1972) provided data for total length weight relationship. Brusher (1972) reported that length of *P. indicus* is linear and positively related.

In this study production period was only four and half months. This time is not sufficient for growth. Generally production period should be six months. One the other hand two month belongs to winter season. In this time the growth rate was slow.

Prawn production rate Thailand in 2338kg/ha (Vicki, 2007), Vietnam 1000-1500 kg/ha (Ridmontri, 2002), China 1500kg/ha (Welmin and Xlanping, 2002), India 600-1000kg/ha (Ralzeda *et. al.*, 2005) where Bangladesh production only 432kg/ha, (Ahmed *et al.*, 2008b) compered to that 336kg/ha reported by (Muir, 2003) with a six to seven months culture period.

In this study the total production of prawn without probiotic was 397kg/ha where as 492kg/ha for only Zymetin treated pond, 582kg/ha for only Super PS treated pond and 656kg/ha for both Zymetin and Super PS treated pond. It is found that there was significant different between probiotic treated pond and non probiotic treated pond. Comparing the current production of our

country (432kg/ha) it is reported that application of probiotic in prawn culture increases the production than the non treated culture system.

It was clear from this study that the application of probiotic, in the water had beneficial effects on the survival rate of Prawn (*M. rosenbergii*). As a result production of prawn also increased. In my present study significant variation was found in growth of prawn between probiotic treated and non treated pond. Average weight of mixed probiotics treated pond was 40.06g and 24.93g for non treated probiotics pond. In the present study the growth rate of prawn was poor in control because of not applying any probiotics.

Significant difference in growth rate was not observed between the control group and the probiotic diet group for estimate the effects of probiotics on *Japanese flounder*. These results are not in agreement with the findings of (Byun, 1997) who concluded that the growth rate of flounder was improved by feeding with a probiotic diet. The survival rate in the probiotic diet group was significantly higher than that in the control group. These are related to our experiment.

The maintenance of good water quality is essential for both survival and optimum growth of culture organisms. The levels of metabolites in pond water that can have an adverse effect on growth are generally an order of magnitude lower than those tolerated by fishes/prawns/shrimps for survival. Good water quality is characterized by adequate oxygen and limited levels of metabolites. The culture organisms, algae and microorganisms such as bacteria produce metabolites in a pond. The major source of nutrients in aquaculture is the feed. Because large quantities of feed are loaded in ponds, excess feed, fecal matter and other metabolites become available in large quantities for the growth of algae and microorganisms.

There was no significant effect of probiotic on the water quality during the study. This result may be explained by the good water quality and laboratory conditions in this study in contrast to theirs. In this study water pH was lies between 7.76-7.85. Concentrations of ammonium and

nitrate ranged 0.4-1.2 and 0.3-1.0 respectively. Almost same result was found by (Boyd and Tucker, 1998), during the culture of *P. vannamei*.

The pH of freshwater ecosystems can fluctuate considerably within daily and seasonal timeframes, and most freshwater animals have evolved to tolerate a relatively wide environmental pH range. pH is a measure of hydrogen ion concentration in water and indicate how much water is acidic or basic. Water pH affects fish metabolism, physiological process, toxicity of ammonia, hydrogen sulphides and solubility of nutrient thereby well-being and fertility. pH at the range of 7.5-8.5 is best for fish growth (Knowlton, *et. al.*, 1984) and can be increase by application of lime. In the total study period pH of the prawn culture pond ranged 7.76 to 7.85, which is suitable comparing to study of *P. vannamei* (Boyd and Tucker, 1998). Agriculture gypsum may be applied to correct alkaline pH. Changes in the pH, especially sudden changes, can prove harmful or even fatal to fish. As the pH rises it increases the toxicity of chemicals such as ammonia. It is an important factor to monitor during the break-in of a new tank. pH changes are particularly hard on young and sick fish. In a number of species of fish, breeding occurs only within a specific pH range.

Salinity plays an important role in the growth of culture organisms through osmoregulations of body minerals from that of the surrounding water. The optimum range of salinity for black tiger shrimp is between 15 and 50 ppt, (Cheng and Liao, 1986) although the shrimp will accept salinity between 5 and 38 ppt. Prawn is a fresh water crustacean. typically; the salt concentration in a fish is about 0.5% higher than its surrounding water. As a result, there is a constant influx of water into the fish through osmosis, diluting its body fluid. In this study period salinity of culture prawn was 0-0.5ppt.

Hardness refers to the amounts of calcium and magnesium in the water and is expressed as ppm of equivalent CaCO_3 . Although freshwater prawns have been successfully raised in soft water (5-7 ppm total hardness), the shell is noticeably softer and may be more susceptible to bacterial infection. To avoid this condition, Optimum hardness for aquaculture is in the range of 50 to 400ppm of hardness. Hardness of pond water can be increased through an application of a source of calcium such as agricultural gypsum or calcium chloride. Under the assumption of 100%

purity, an increase of 1.00 ppm in total hardness can be achieved with an application rate of 1.72 ppm (Watson, 1996). In the study period hardness ranged to 300–450 mgCaCO₃/L, which is slightly higher than optimum level, and there was no significance variation among the treatment. Higher calcium concentrations are desirable because calcium also protects fish from ammonia and metal toxicities (Wheaton, 1977).

Too much hard water can also be a problem. Therefore, Wetzel (2001) recommended a range of hardness from 50 to 200 mg/L, which is ideal for freshwater prawn culture. But the present study demonstrated that the growth of the prawn was better above 300 mg/L bicarbonate alkalinity level. Bartlett and Enkerlin (1983) reported that hardness levels between 940 and 1060 mg/L CaCO₃ did not adversely affect the prawn growth; but their water had relatively low alkalinity of 58–86 mg/L CaCO₃.

Alkalinity is a measure of the capability of water to neutralize acids. Alkalinity is expressed as ppm equivalent CaCO₃/L. In most natural waters, the predominant bases are bicarbonate and carbonate. Thus the present study indicated that the freshwater prawn, *M. rosenbergii* is sensitive to higher levels of water calcium and higher levels of bicarbonate alkalinity as they affected both survivability and growth. Ideal range from 60–300 ppm as CaCO₃ and it can be treated with lime. Less than 20 ppm leads to fluctuation and more than 300 ppm may become unproductive due to limitation of carbon dioxide availability (Colt and Bouck, 1984). Alkalinity of the present study ranged to 180–240 mgCaCO₃/L which was suitable for prawn growth. It is also found that application of probiotic did not affect water alkalinity and hardness significantly. The present findings are also supported by New and Singholka (1985) whose recommended levels were between 40 and 100 mg/L CaCO₃ for successful farming operation and 100–180mg/L CaCO₃/L is for culture. Low calcium content has often been considered as a constraint to freshwater crustacean's distribution, but in case of *M. rosenbergii*, the effect of high hardness water appears to be more of a handicap, particularly when carbonate alkalinity is also elevated (Brown *et al.*, 1991).

Aquaculture organisms are cold-blooded animals. They can modify their body temperature to the environment in normal condition, unlike the warm-blooded animals, which can react to maintain

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the optimum body temperature. All specimens that were held at freshwater and 24 – 29 °C did not show any significant difference in total length, carapace length and weight of females except at 34 °C where they decreased significantly.

The optimum range of temperature for the Black Tiger shrimp is between 28°C-30°C. Increase in temperature beyond 30°C increases the activity level and the metabolism. This also increases the growth rate. If the temperature still increases then the shrimp reaches a threshold of physical and nutritional tolerance, which is 33°C in poor quality water or 35°C in good quality water and remains stationary at the pond bottom. If the temperature falls below 20°C, the metabolism reduces and the shrimp will take less feed. Shrimps cannot tolerate a temperature less than 13°C.

In this study culture period was August to December. In August to October water temperature of culture pond ranged to 25-30°C, and prawn shown high growth rate, But at November and December water temperature was lower ranged to 13.5-18.5 °C. This study results showed that growth of the prawn increased as temperature increased from 24 to 29 °C, while growth declined to decreasing with temperature. Firkins and Holdich (1993) reported similar results and stated that growth of crayfish decline at 34 °C might result from increased metabolic demands approaching the calorific intake, leaving little energy for growth, despite animals being fed to excess.

The optimum dissolved oxygen (DO) content of pond waters is in the range of 3-5 ppm saturation level (FAO, 2001). Aeration is a proven technique for improving DO availability. Any sort of agitation improves the DO content and among which paddle-wheel, aerators aspirators are most common. Oxygen deficiency creates hypoxia. According to Herreid (1980), the mechanisms involved in response to the hypoxia depend on the environment and the physiological state of the animals. Temperature, salinity, pH, pollutants, activity and size, among others, are the factors that determine changes in the metabolism of animals exposed to hypoxia.

In the present study dissolved oxygen (DO) concentration in water was 4.5-5.2 ppm. So there was no significant affect of probiotic on culture of prawn. According to Herreid (1880) oxygen

Transparency is related to turbidity, or to the quantity of suspended materials. For *M. rosenbergii* culture, it has been recommended transparency values ranging from 25 and 60 cm (Boyd & Zimmerman, 2000; New, 2002). In this study water transparency ranged 40-60 cm. This is closely related with Boyd & Zimmerman, 2000; New, 2002.

Pond bottom soil pH can range from less than 4 to more than 9, but the best pH for pond soils is considered to be about neutral (Boyd, 1995). Maximum availability of soil phosphorus usually occurs at about pH 7. Most soil microorganisms, and especially soil bacteria, function best at pH 7 to 8. Acid ponds do not respond well to fertilization and liming is the only way to improve water quality with acid soil and it is the soil that must be corrected for lasting effect, rather than the pH of the water. In the present study soil pH remained almost similar among the treatment that was ranged from 6.7-7.7.

Ponds sediment plays an important role in nutrients cycling by retaining or releasing nutrients. Moriarty (1996) strongly advocated the use of probiotics amendments in aquacultural pond. Suhendra *et al.* (1997) found that routine use of commercial probiotics in a shrimp farm in West Java resulted in reduced organic matter accumulation, improved water quality and enhanced environmental conditions. Our data showed the standard concentrations of TP (0.02-2.67 %), OC (1.067 to 1.32 %), Ca (0.13 to 0.17%), Mg (0.08-0.09%) in sediment after the ponds treated with commercial probiotics which is strongly related to the study with Suhendra *et al.* (1997)

It is difficult to assess organic matter concentrations in soil because there are different kinds of organic matter. Organic soils have high concentrations of often recognizable plant remains that are quite resistant to decomposition by bacteria and other microorganisms. Organic soils have 15 to 20% organic carbon (about 30 to 40% organic matter). Such soils are not good for pond aquaculture and should be avoided. Soils with less organic matter are known as mineral soils, and as previously discussed, organic matter in mineral soils is considered to be labile if microorganisms can decompose it readily or refractory if it decays slowly. Methods of soil organic matter analysis do not distinguish among coarse plant remains, refractory organic matter,

and labile organic residues. They only measure the total concentration of organic matter. Thus, a soil with 10% organic matter may be perfectly acceptable for pond aquaculture (Boyd *et al.*, 2002).

Successful aquaculture relies on adequate microbial control to prevent the proliferation of pathogenic bacteria that often results in fish mortality. Bacteria can accumulate in fish by ingestion or through the body surfaces and is transported by the body fluids into the tissues. This may be one of the causes of indigenous microflora formation and bacterial infection in fish. Microorganism is one of the important parameter for successful growth. Gram *et al.* (1999) reported a broader range of live microbial supplement (probiotics) which beneficially affects the host animal by improving its microbial balance. In the present investigation, the probiotic-treated Prawn culture pond water and sediment showed lower bacterial load (3×10^4) than the control pond water and sediment (5.3×10^5). These results indicate that probiotics reduced bacteria in the prawn culture pond water and sediment.

Prawn is an omnivorous species. Various types of feed are used in the study area but most farmers are found to use homemade feed e.g. cooked rice, rice bran, oil cake; which is different from Barmon *et al.* (2006) and Ahmed *et al.* (2008b) who reported snail-meat used commonly in prawn farming system. Similar to Barmon *et al.* (2006), the farmers in the study area do not maintain any standard feeding system but apply trial-error techniques. In present study we used MEGA feed which contain 34% protein. This level of protein is suitable growth and profitable to farmer.

CHAPTER 6

CONCLUSION

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Conclusion

Though Bangladesh shrimp and prawn production is increasing day by day because of inland fisheries but the amount of shrimp and prawn from our natural water bodies is declining because of overfishing. With increasing demand of fish and for environment friendly aquaculture, the use of prebiotics in aquaculture is now considered as alternatives to antibiotics and chemicals to improve the quality and sustainability of aquaculture production. On the other hand probiotic is environment friendly and can produce antibiotic and disease free quality prawn. Not only higher production but also quality prawn product is very much concern to attract the export market. It was found that both zymatin and super PS treated pond showed more production. By applying probiotics it could be possible to grow more prawn than the control pond with same input like quality feed, stocking density, water supply etc. It can be possible to get more benefit from one ha pond using probiotic. Prawn has wide range of culture area from coastal to any freshwater reservoir over the country. As a developing country prawn has diverse contributions such as food production, employment opportunity for income-poor fisher-folks, and valuable foreign currency earnings. If the findings of this study will extent to the farmer and prawn culture the total production of prawn will increase.



CHAPTER 7
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Chapter 7**References**

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APPENDIX

Table I: Available types Shrimp Probiotics

SUPER PS		M-BACTO	
	<p>Super Photosynthetic Bacteria is a Phototroph bacteria composition which is effective to solve abundance of organic matter problems at the bottom of shrimp ponds.</p>		<p>A probiotic product which consists of Bacillus sp. It is useful for creating optimum pond water quality in cultivation. It optimizes the balance of pond cultivation ecology and maintaining plankton stability.</p>
BIO SOLUTION		BI KLIN	
	<p>Bio Solution consists of Bacillus sp bacteria. This product has the benefits of eliminating bacterial Pathogen domination (such as Vibrio sp.), deteriorated shrimps, maintaining the cleanliness of pond bottoms, also increasing shrimp activity and appetite.</p>		<p>Bi Klin is specially probiotics made to overcome digesting track complications in shrimps. It is beneficial to eliminated pathogenic bacterial (such as Vibrio sp.) in Hepatopankreas, also improving shrimp's immunity.</p>
SUPER NB		SUPER VAMEI	
	<p>Super NB is intended to create the optimum shrimp pond ecology and aid development, also minimizes the risk of diseases in shrimps.</p>		<p>Super Vamei is a synergy combination between Rhodobacter sp and Bacillus sp which are becoming the strong decomposer of organic materials. This product is suitable for vannamei cultivation.</p>



Photo gallery



Experimental pond before digging and dike repairing



Experimental pond during bottom mud removing, digging and dike repairing



Experimental pond after digging and dike repairing

Photo gallery



Experimental pond before digging and dike repairing



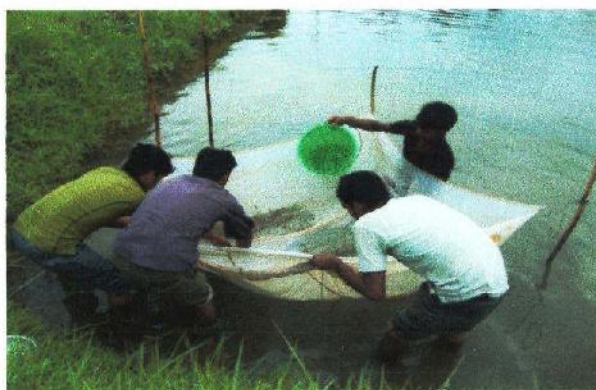
Experimental pond during bottom mud removing, digging and dike repairing



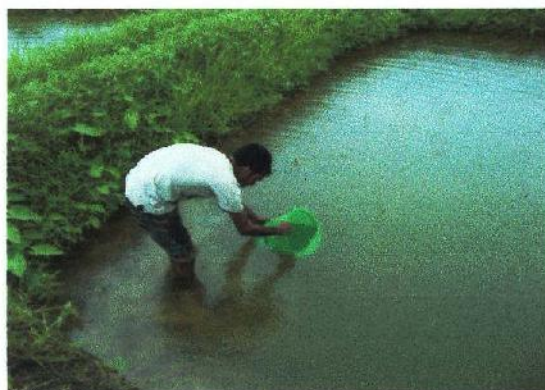
Experimental pond after digging and dike preparing



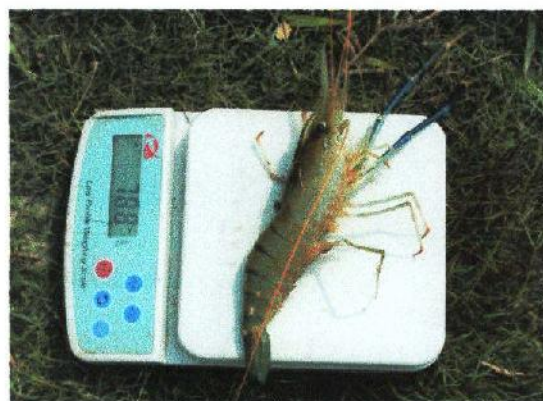
Experimental pond are ready for stocking



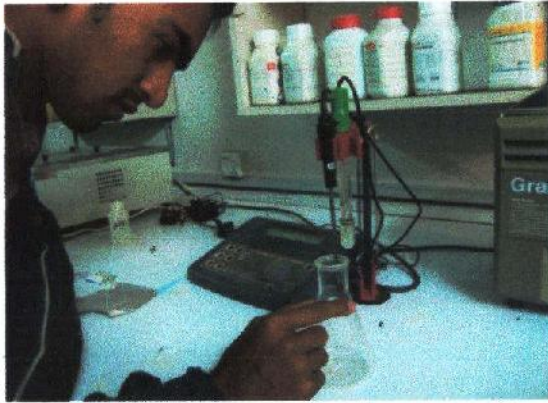
Juvenile collection from nursery pond



Juvenile stocking in grow out pond



Measuring length and weight of prawn



Water and soil parameter measurement at laboratory

Final Harvesting

