Stocking Density and Male Morphotypes Formation of Prawn (Macrobrachium rosenbergii) in Probiotic Based Culture System

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Session: 2013-2014

Fisheries and Marine Resources Technology Discipline

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A Thesis

By

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Declaration

Stocking density and male morphotypes formation of prawn (*Macrobrachium rosenbergii*) in probiotics based culture system

The results presented in the 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

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November, 2015

DEDICATED TO

My Respectable Parents, who sacrificed for me, inspired and blessed me in every way of my life

Acknowledgement

All the praises are due to the almighty Allah who has guided the author with the blessing to complete this project thesis for the degree of M.Sc. in fisheries from Khulna University under the Fisheries and Marine Resource Technology Discipline.

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I also convey thanks to my friends whose inspirations were all along with me in the hard time during the course of the present study.

Last of all, I express my deepest gratitude to my parents and my family members for standing all the way in my side.

Sadia Ashrafi

140624

Stocking Density and Male Morphotypes Formation of Prawn (*Macrobrachium rosenbergii*) in Probiotics Based Culture System

Abstract:

Formation of different morphotypes in male galda in culture ponds is a potential drawback in increase of production; the males with blue claws are bigger than either the orange claw males or SM males. The proportion of these morphotypes in cultured population determines the total production obtained and needs to be looked at what trigger the formation of the different morphotypes. With this aim in view the present study was conducted in an on-going experiment based on the impact of two commercial probiotics – Zymetin (a food related probiotics) and Super PS (a soil related probiotics) on the production of galda, stocked in three densities. Efforts were made to see if the sex ratio in stocked galda and the doses of probiotics used in the culture treatment had role in the formation of different morphotypes with consequent net result in production obtained; more males with BC claws result in higher production. Three stocking densities tested with the two probiotics in the experiment were - 2 juveniles /m^2 (T_1), 3 juveniles/m^2 (T_2) and 4 juveniles /m^2 (T_3). The sex ratios obtained in the final production were found to be 1.08:1(T_1), 1:1.13 (T_2) and 1.78:1 (T_3) and proportions of BC, OC and SM males were obtained to be 57%,35% and 8% (T_1); 64%, 31% and 5% (T_2) and 25%, 44% and 31% (T_3). The highest proportion of BC male (64%) was obtained from T_2 treatment with consequent highest production of 1158kg/ha followed by 57% BC male with total production of 667.87kg/ha in T_1 and 25% BC male with total production of 787.18kg/ha in T_3. Evidently in T_3 the proportion of SM male (31%) led to increase of production than in production obtained in T_1 with next higher proportion of BC male (57%). The results of the experiment suggested that stocking density of 3 juveniles/m^2 (5g Zymetin with 20g Mutagen/kg feed) and Super PS (15l liter Super PS with 7.5kg sand /ha/week) was the best in galda production with slightly more females in the production ponds leading to higher proportion of BC males. The findings obtained can be regarded as a preliminary one; further researches can be carried out in this regard in future.

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homogeneous. By contrast, the males show heterogeneous growth and therefore can be differentiated into three distinct morphotypes, viz., small males (SM), orange claw males (OC), and blue claw males (BC). Blue clawed (BC) males are relatively large and possess long claws with a high claw to body length ratio. Orange clawed (OC) males are also relatively large but possess smaller claws with a lower claw to body length ratio. Small males (SM) are considerably smaller and possess short delicate claws with the lowest claw to body length ratio. The three male morphotypes represent three developmental stages of the male maturation process and are known to undergo transformation from SM → OC → BC. SM occupies the initial stage of the developmental pathway. SMs are subordinate, OC stage is subdominant and represents a stage of high somatic growth. BC stage is dominant. In a usual phase, SM transforms to OC and then to BC, but once a set of prawns reach the terminal morphotype it inhibits the transformation of other small males to successive stages (Ra'anan and Sagi 1985). The dominant BC capable of drawing a territory of its own is termed "bull" and the undersized suppressed SM is termed"runt" (Karplus *et al.* 1992). This complex structure that is basically confined to males of *M. rosenbergii* is termed "heterogeneous individual growth" (HIG). HIG is one of the limiting factors for uniform growth and development of *M. rosenbergii*, in the grow out system. In farms, generally, about 50% of the male population comprises the SM having a weight range of 5-20 g, while OC forms about 40% which is characterized by a wide weight range of 30-180 g, whereas BC forms only 10% representing the highest weight class and reaching up to 250 g. Hence, the economic viability of farming this species is profoundly influenced by the relative proportion of OC and BC morphotypes and their allied intermediary stages in the harvested population.

In general, the heterogeneous growth shown by the males of *M. rosenbergii* has been associated with intrinsic factors such as genetic differences, hatching order or age of metamorphosis (Smith *et al.* 1978,). Stocking density is one of the most important extrinsic factors for the growth performances of different culture species. Because increase in stocking densities give rise to competition for limited space and food (Magnuson 1962), which significantly affects the size, weight, survival rate of the culture species. So it may be an important reason for having three male morphotypes in different quantity in culture program. The ratio of male and female may also be an important factor in this case. If one male mate with several females, there will be

competition at the time of breeding, which may causes HIG. The wide disparity in size structure of the cultured stock and the skewness in their weight distribution, which is profoundly influenced by the male morphotypes, apparently appear to be a commercial disadvantage of culture of this species. Because consumers always prefer larger and healthy fishes. So, to get the best output from the galda culture, the heterogeneous growth should be minimized.

Disease is considered as a serious anxiety in both aquaculture and capture fisheries due to the anthropogenic and environmental stresses, and the spread of pathogen by the different activities in aquaculture (Naish *et al.*, 2007; Liao and Chao, 2009). The utilization of probiotics in aquatic environment has been increasing as an alternative of antibiotics and to fulfill the demand of eco friendly and sustainable aquaculture practices (Gatesoupe, 1994). Probiotics are non pathogenic live microorganisms which prevent the colonization power of pathogenic microorganisms as well as provide the preventative action of some diseases. According to the definition by Fuller (1992), probiotics are living microbes which are used in feed supplements and influence the host beneficially by developing the intestinal balance of microorganisms. Probiotics such as *Bacillus spp.* and lactic acid bacteria which are called 'bio friendly agents' are used in aquatic culture system to prevent and to fight with harmful microbes as well as to increase the growth of target species. The probiotics treated farming method is conducted by application of commercial probiotics containing beneficial bacteria such as *Clostridium butyricum, Streptococcus faecalis, Bacillus mesentericus, Rhodobacter sp* etc. The probiotics are being marketed commercially in three groups namely Super PS and Super Biotic (Soil Probiotics), pH fixer (Water probiotics) and Zymetin (Nutrient probiotics). The effectiveness of probiotics has got a big attraction and acceptance for producers and consumers due to its efficiency, lower cost, lower side effects for health and easily usable to farmers.

For successful culture of *M. rosenbergii*lies, standardered stock density, use of quality seed, application of proper probiotics and management procedures both prior and after stocking is needed. In present study, three different stocking densities (2, 3 and 4 juveniles/m^2) with probiotics named Zymetin and Super PS were tried to find out its effect on the proportional formation of male morphotypes as well as growth performances and production of prawns. It

was found that stocking densities and probiotics has significant effect on the HIG, growth and production of freshwater prawns; and stocking density of 3 juveniles/m^2 with proper dose of Zymetin and Super PS can be beneficial to the culture of *M. rosenbergi.*

Objectives:

- To find out the relationship between stocking density and male morphotypes formation in prawn.
- To find out the effect of sex ratio in the formation of different morphotypes in male prawn

Chapter

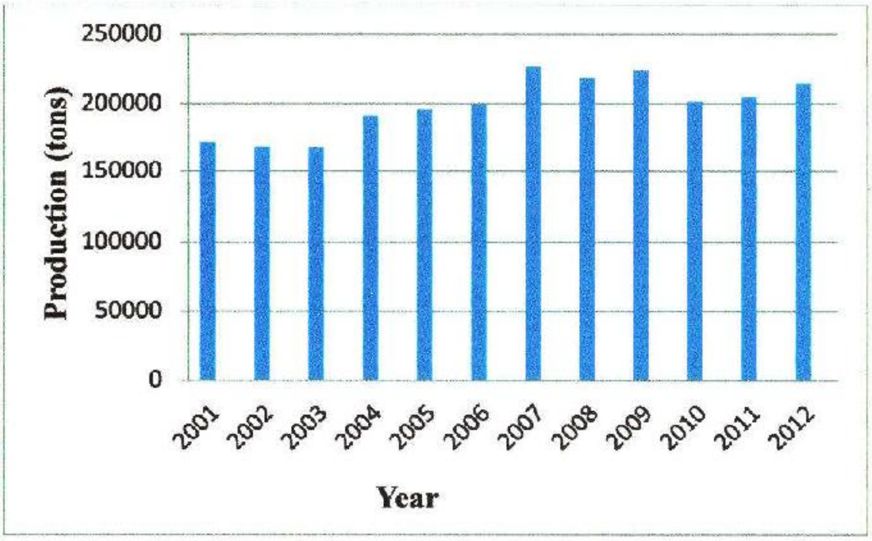
Two

LITERATURE REVIEW

Chapter 2: Review of literature

2.1 Present status of prawn aquaculture

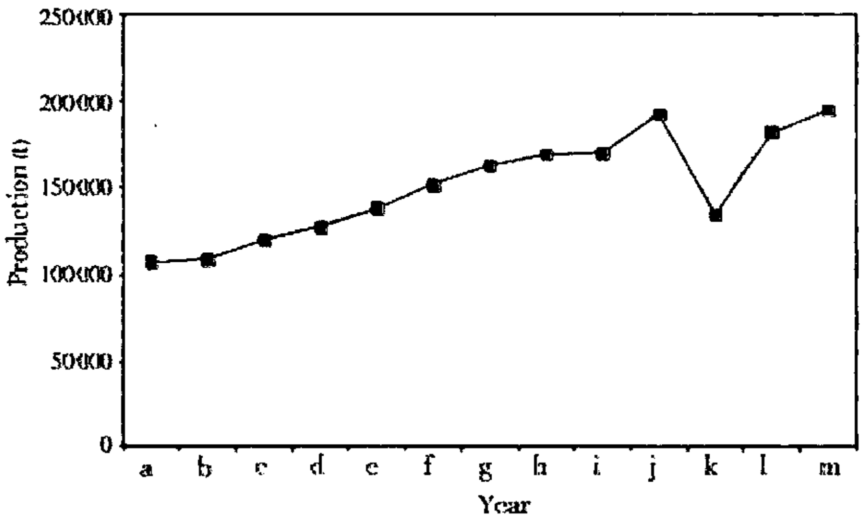
The first recognised output of farmed giant river prawn (*M. rosenbergii*) production recorded in FAO statistics appeared in 1970 (FAO2008), showing a production of 10 t by Mexico and less than 0.5 t by Mauritius. By 1979 annual global production had risen above 1100 t; farming statistics were being reported to FAO by Brazil, Ecuador, French Polynesia, Guam, Honduras, Malaysia, Mauritius, Mexico, Myanmar, Thailand and the USA. A major expansion in the global output of farmed freshwater prawns first became obvious in the mid-1990s when China began to submit separate statistical returns for farmed *M. rosenbergii* (FAO 2008), showing that Chinese production had already exceeded 37 000 t by 1996. Expansion in India was even more explosive than in China, rising to a peak of 42 800 t in 2005. The other major producing countries of *M. rosenbergii* are Thailand, Taiwan and Vietnam. Following figure shows worldwide production of *M. rosenbergii* from 2001-2012.



(Source : FAOstat, 2014)

Fig 1 : Worldwide production of *M. rosenbergii* .

In Bangladesh, freshwater prawn farming first started in the southwest region in the early 1970s (Mazid 1994). The expansion of prawn cultivation has been dramatic, and since1990 adoption has accelerated, By 1998, about 46% production of M. rosenbergii still came from the capture fisheries with 84% production of the overall production of the Macrobrachium species. A recent statistics in 2011-2012, indicated that 70% of the total production of freshwater prawn was from aquaculture while only 30% was from capture fishery. In Bangladesh, more than 70% of the prawn farming area is located in south western region. Prawn culture area has been increasing quickly in current years where the average growth increment is 15% per year (Khondoker, 2007). Production from 1999 to 2012 have shown in figure . From 2001-2012, the exported quantity has increased from about 300000 to about 50000 t. Bangladesh is now considered as one of the major prawn exporting countries .



(Source: DOF , 1013)

Fig :2 Total production of freshwater prown in Bangladesh n different years. *a: 1999-00, b: 2000-01, c: 2001-02, d: 2002-03, e: 2003-04, f: 2004-05, g: 2005-06, h: 2006-07, i: 2007-08, j: 2008-09, k: 2009-10, l: 2010-11, m: 2011-12).*

2.2 Culture Techniques of prawns

Several factors are reported to be affecting the production, especially growth and survival in freshwater prawn farming worldwide (New 1995). The stocking rate is found to be a major factor affecting the individual growth of prawns (Karplus et al. 1986). If the correct stocking densities are not practiced in fish culture system, the fry even with excellent health condition cannot perform satisfactory growth (Sanches & Hayashi 1999). The growth rate and the stocking density have an intricate relation, as the correct stocking density provides sustainable aquaculture through proper uses of feed, obtaining highest production, keeping environment good and healthy fish. Most of the time higher stocking density creates negative impacts on production than lower density creating competition for food and shelter causing increased risk of disease outbreak (BFS 2011).

The stocking density for prawn cultivation ranged from 1 to 10 PL/m^2 in many different parts of the world (Nagarathinam et al. 2000) and in India the stocking density of *M. rosenbergii* varied from 2 to 5 PL/m^2 (Stephen 1997). At present, in Bangladesh traditional culture system, extensive culture system, improved extensive and semi-intensive culture system are being practiced on the basis of stocking density of PL (post larvae) and the management technique during the culture period. In traditional culture system no strict stocking density is followed and completely depends on the open entrance of PL from the natural sources through tidal waters (mainly in *gher* system). The stocking density in extensive culture system fluctuates from 1 to 2 PL/ m^2 and no artificial feed is used; only the organic and inorganic fertilizers are used to enhance the plankton production whereas in improved extensive culture system supplementary feed (which is made by locally available ingredients) is used along with extensive system. In semi-intensive system, however, the stocking density varies from 2 to 3 PL/ m^2 and fully depends on the artificial feeds (Ahamed et al. 2014).

2.3 Production performance based on stocking density

According to Wohlfarth et al. (1985), higher net production is achieved due to the higher stocking density of prawn. Nagarathinam et al. (2000) conducted an experiment using two stocking densities at 5 and 10 PL/m^2, where he found the higher growth rate, higher specific growth rate and lower FCR for the lower stocking density (5/m^2). In Bangladesh most of the farmers are used to culture of prawn with a lower stocking density in the ponds and are not getting higher production. So to get the better production it is important to use the optimum stocking density of prawn in pond culture system.

Similar experiments were done in case of tiger shrimp *Penaeus monodon* and green tiger shrimp *Penaeus semisulcatus*. The results show significantly negative correlation between individual growth and density. It was reported that, stocking density of 15 PLs/m^2 of green tiger shrimp and

20 % water exchange rate exhibited the highest net profit and would seem to be the most desirable density and water exchange rate in the system studied (Gaber et al. 2012).

2.4 Biology of *M. rosenbergii*

2.4.1 Taxonomy

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Order: Decapoda
Family: Palaemonidae
Genus: *Macrobrachium*
Species: *Macrobrachium rosenbergii*

2.4.2 Habitat and distribution

Macrobrachium rosenbergii is indigenous to South and Southeast Asia, northern Oceania, and the western Pacific islands. This species has been transferred from their natural location to many parts of the world, initially for research purposes and ultimately for commercial production Freshwater prawns require brackish water in the initial stages, but most of their lifecycle is spent in turbid, riverine systems. They are not cold tolerant. Gravid females migrate downstream into estuaries, where eggs hatch as free-swimming larvae in brackishwater.

2.4.3 Anatomy of *M. rosenbergii*

The body of this species is composed of three parts; head named carapace, abdomen and tail. There are five pair of walking legs at the head part and five pair of swimming legs at the abdomen with one pair at each abdominal segment except the last one. First pair walking legs is used in putting food into the mouth. The second pair is larger than others and ends in pronounced claws which are used for self defense and catching foods. The rostrum develops at the tip of the carapace. Tail part is composed of two uropods and one telson.

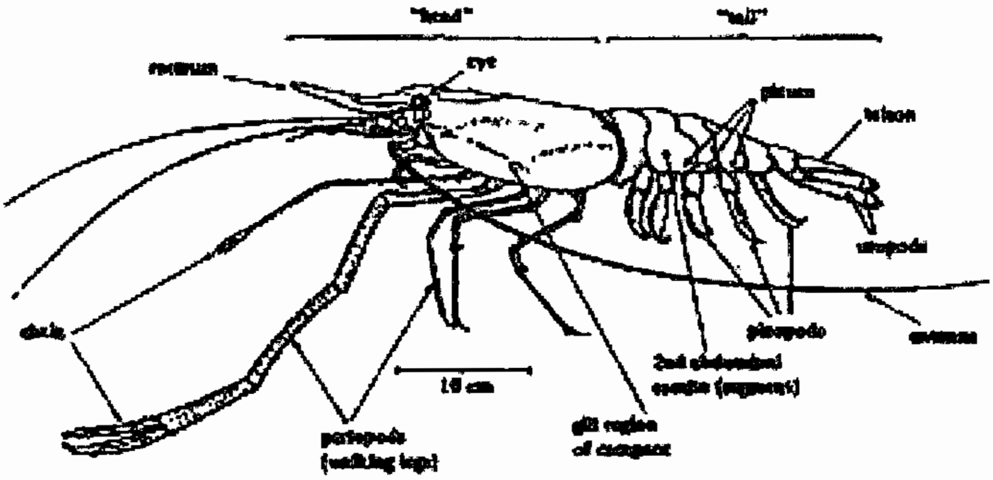


Fig :3 Anatomy of *M. rosenbergii*

2.4.4 Growth and life cycle

Freshwater prawns have a hard outer shell that must be shed regularly in order to grow. This process is called "molting". Because of these periodic molts, growth occurs in increments, rather than continuously. This results in four distinct phases in the life cycle; egg, larvae, post larvae, and adult.

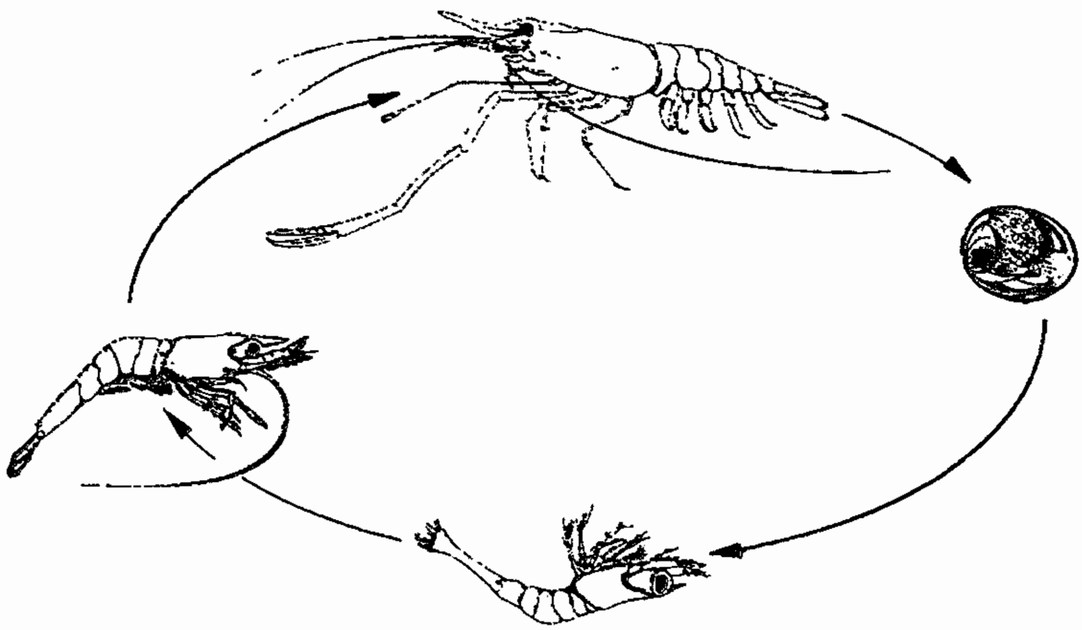


Fig : 4 Life cycle of Egg *M. rosenbergii* shows egg (incubated by female for 21-25 days) , zoea(5 to 12 instars in 20 to 40 days) , post larva/juveniles to maturity (120 -210 days)

2.4.5 Food and feeding

Larvae mostly consume zooplankton (mainly minute crustaceans), very small worms, and larval stages of other crustaceans. Post larvae and adults are omnivorous, eating algae, aquatic plants, molluscs, aquatic insects, worms and other crustaceans.

2.5 Growth patterns of *M. rosenbergii*

In freshwater prawn farming, Heterogeneous Individual Growth (HIG) has been a common phenomenon. When high stocking density monoculture is practiced; the yield obtained usually consisted of prawns of wide size range (Smith et al. 1978; Cohen et al. 1983). Ranjeet & Kurup (2002) discussed about the concept of HIG, factors underlying HIG and its implication on culture. They found that at lower densities (1.5 and 2.5 PL/m^2 the proportion of undersized prawns SM were comparatively low. Likewise, the percentages of larger Blue Clawed (BC) Males increased in these treatments. Opposite result was found at higher stocking densities (4.5 and 6 PL/m^2). They also observed maximum survival rate at least stocking densities.

Patricia et al. (2004) examined the effect of intensification on growth, survival, productivity, population structure, and distribution of harvested biomass in individual size classes of *M. amazonicum*. The result showed that, *M. amazonicum* tolerates grow-out intensification and may be raised in both semi-intensive and intensive systems stocked at very high densities yielding high productivity. But average individual weight significantly decreased and increased as stocking density increased, while survival was not affected.

Various management approaches designed to minimize HIG have concentrated on selective harvesting or size grading of pond populations of prawns. Malecha et al. (1981) proposed a management practice that included pre-harvest size grading and restocking of non-market size prawns after complete harvesting. Other studies have evaluated the potential for increasing yield by size grading nursery populations prior to stocking. (D'Abramo et al. 1989) and also by resorting to stock of size graded post larvae (Karplus et al. 1986, 1987). Stock manipulation has been routinely practiced for the husbandry in order to optimize production. Apart from this initial screening, attempts have also been made to standardize the stocking density at different stages of culture. The duration, pattern and stock size during the nursery phase have also been subjected to change in order to increase the retrieval rate. With the advent of cull harvest techniques, farmers can now market the prawns depending on the weight class and correspondingly improve revenue.

2.6 Formation of male morphotypes

Patricia et al. (2004) investigated the occurrence of morphotypes in *M. amazonicum* males. Four distinct morphotypes were identified: Translucent Claw (TC), Cinnamon Claw (CC), Green Claw 1 (GC1) and Green Claw 2 (GC2). They differed in cheliped morphology and some morphometric relationships. Chelipeds were translucent in TC prawns while in CC they were generally cinnamon-color. Both showed a few spines and some low prominences similar to very small tubercles. GC1 and GC2 showed long moss green chelipeds provided with long and robust spines.

In Andean river shrimp *Cryphiops caementarius* two morphotypes can be distinguished based on the differences in the allometric relationships between several morphologic traits and carapace length. The 'dominant' morph is characterized by stout teeth on the cutting edges and by dark blue color on the external surface of the major cheliped. During competitive interactions, large males fight more vigorously than small males and, consequently, it is expected that they accumulate more injuries (Rojas et al. 2012).

Genetic differentiation among BC, OC and SM male freshwater prawn were found by Banu and her fellow researchers (2015). The total numbers of alleles per locus in the BC, OC and SM groups were found to be 23, 27 and 21 respectively. The average observed heterozygosities (Ho) ranged from 0.48 to 0.56 across all loci in the three groups. Pair-wise comparisons and the FST values revealed significant genetic differentiation across all groups. The greatest genetic distance was observed between the BC and SM groups and lowest one was between the BC and the OC groups.

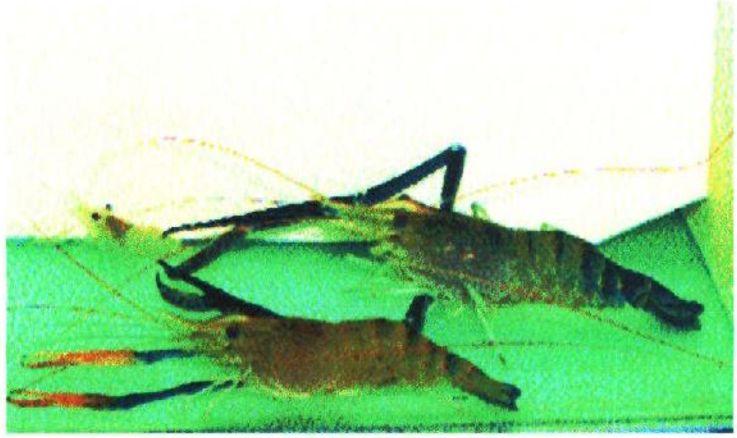


Fig: 5 Three morphotypes (BC, OC, SM) of male freshwater prawn.

2.7 Application of probiotics

According to Nabil and his colleagues the optimum stocking density of postlarvae *M. rosenbergii* laid between 50 to75 PL/m^2 and increasing the stocking density more than75/m^2 had negative effects on growth performance parameters. Each group of treatment fed on a diet without probiotic and with the tested probiotic (Ecobiol-aqua which contains spores of a strain of *Bacillus amyloliquefaciens*) supplemented to the diet at 0.01 % level. Probiotic supplementation had insignificant effects on final weight; total weight gain; specific and relative growth rates, regardless of stocking density. Within each stocking density supplementing the diets of freshwater prawn with probiotic improved the survival rate and the improvements were more pronounced at the lower stocking densities.

Though there are many research reports on the uses of probiotics in fish culture (Zhou et al. 2009; Hai et al. 2009; Li et al. 2006), a very few studies are available regarding the fish culture especially prawn culture with probiotics in Bangladesh. A very few researchers have conducted investigation on shrimp culture with probiotics in Bangladesh with respect to production and stocking density (Hossain et al. 2013; Ghosh et al. 2013). Saad et al. (2009) from Egypt reported that growth performance of *M. rosenbergii* larvae was enhanced by the inclusion of Biogen® probiotics (2 to 3 %) with feed. Growth of *M. rosenbergii* was improved significantly when they were fed with *Bacillus* KKUO2 and *Bacillus* KKUO3 mixed feed in Thailand (Deeseenthum et al. 2007). A research conducted by Rengpipat et al. (2000) revealed that the immune capacity and disease resistance power of *P. monodon* was increased against *Vibrio harveyi* by using *Bacillus* S11 mixed with feed. It has been reported that feed with Gram-positive and gram-negative probiotics has an effect to increase the amount of lymphocytes, erythrocytes and macrophages that increased lysozyme activity (Gatesoupe 2007). Application of *Bacillus* spp. as probiotics enhanced different water quality parameters, survival rate, growth rate and the health quality of *Penaeus monodon* in juvenile stage and decreased the harmful vibrios (Dalmin et al. 2001). It has been observed from another research that the concentration of P and N can be reduced in the *P. vannamei* culture ponds by using commercial probiotics that increase the production of shrimp also (Wang et al. 2005). Application of *Lactobacillus* spp. and *B. subtilis* BT23 has an effect for controlling *vibrio* spp. and White Spot Syndrome Virus (WSSV) disease in *P. monodon* (Phianphak et al. 1999; Vaseeharan & Ramasamy 2003). Probiotics could be introduced into the aquaculture system through artificial feed or live food such as *Artemia* and rotifer, or by immersion or injection of probiotics organisms (Einar 2002; Irianto & Austin 2002).

Chapter

Three

MATERIALS AND METHODS

Chapter : 3

Materials and Methods

3.1 Study period and study area

The experiment was conducted in the pond complex of Fisheries and Marine resource Tecnology Discipline, Khulna University. The duration of study period was August to December, 2014.

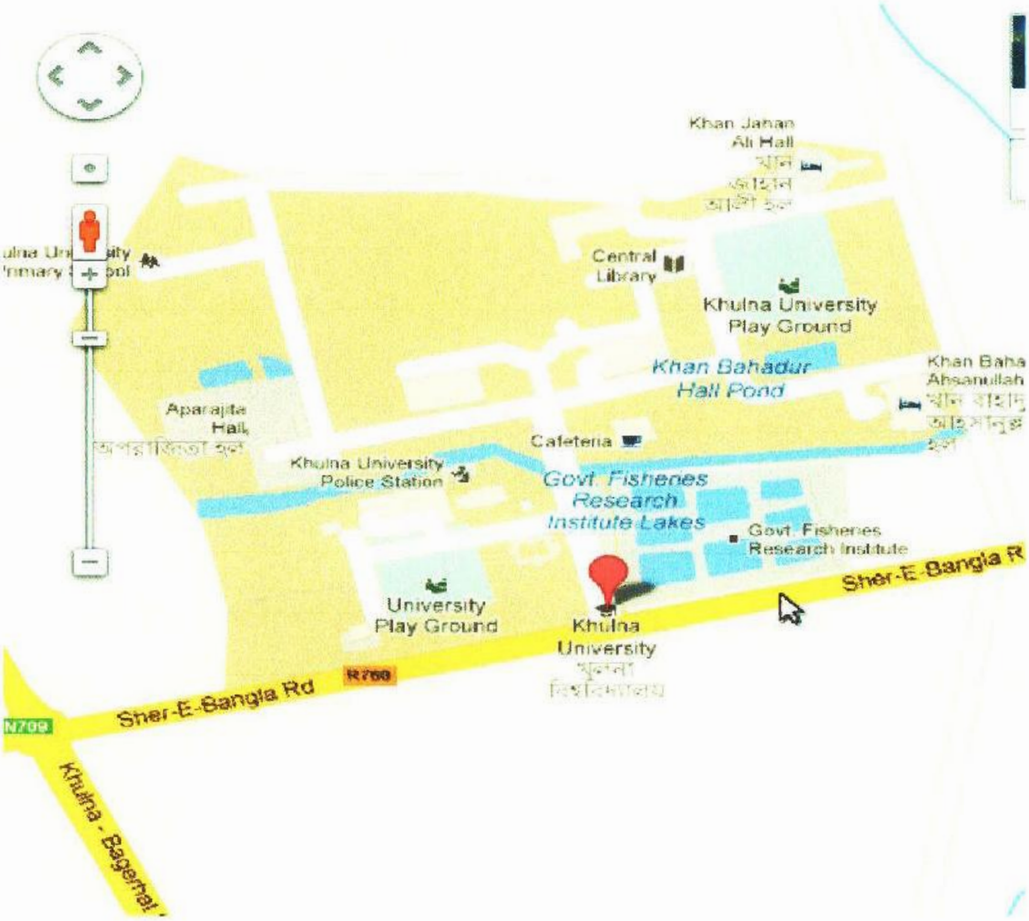


Fig: 3.1 Map of Study area of the experiment.

3.2 Experimental animals

The experiment was conducted growth of giant freshwater prawn (*Macrobrachium rosenbergii*) together with some commercial probiotic bacteria stocked at different densities. The animals were collected from Shrimp Research Station of Bangladesh Fisheries Research Institute, Bagerhat. After certain period of rearing male morphotypes formation was analyzed.

3.3 Experimental design

The experiment was conducted with stocking of juvenile prawns at three different stocking densities. Each of the stocking rates was termed as a treatment and each of the treatment was triplicated. Treatment-1 (T_1) was carried out with a stocking density of 2 juveniles/m^2; Treatment-2 (T_2) and Treatment-3 (T_3) were carried out with stocking densities of 3 and 4 juveniles/m^2 respectively. In addition, all the treatments were implemented with the same probiotics only at the grow-out phase of the culture. Two commercial probiotics Zymetin (a feed probiotic with the bacterial composition of *Streptococcus faecalis*, *Bacillus mesentericus* and *Clostridium butyricum* (1.10×10^8 $FCUg^{-1}$) with wheat flour as carrier (Advance Pharma Com., LTD., Thailand) and Super PS (a soil probiotics with bacterial composition of *Rhodobacter* sp. and *Rhodococcuss* sp. in 10^9 $CFUml^{-1}$) (CP aquaculture, India) were used. The probiotics were applied as 5 g Zymetin with 20 g Mutagen per kg feed in every four days in a week and 15 liter Super PS with 7.5 kg $Sandha^{-1}week^{-1}$. During five months culture period male morphotype formation of animals were studied to find out the percent of BC, OC and SM as well as the production of the ponds under the treatments.

3.4 Pond preparation

Nine experimental ponds were selected for the cultivation of prawns under three treatments. Before starting the experiment all the steps of pond preparation were done carefully with the optimum level of preparation and application measures.

3.4.1 Dewatering and drying

The ponds were first dewatered and dried in the sun for one month to kill all the unwanted animals, germs and pathogens. Excess soils from the bottom were also removed.

3.4.2 Liming

After dewatering, liming ($CaCO_3$) was done at a rate of 250 kg/ha on the bottom of ponds. The ponds were watered by a pump up to a level of 1.5 m.

3.4.3 Unwanted species removal

After watering, rotenone was applied at the rate of 6 kg/ha/ft of water depth to eradicate the unnecessary organisms. The total area of the ponds was finally fenced with a net to prevent entering of unwanted organisms.

3.5 Measurement of water quality parameters

During the study, different water quality parameters *viz.* temperature, pH, DO, salinity, alkalinity, hardness, ammonia and nitrite were measured. These parameters were considered as the important factors to give the better environment for shellfish.

Temperature was measured weekly using digital thermometer (DIGITAL THERMOMETER, made in China, model no WT-2, Temperature range -20 to 80 °C). Salinity of the pond water was measured by Refractometer (ATAGO CO. LTD, Japan, model no, Master- T 2312, Salinity range 0-100 ppt). In this experiment, pH, DO and hardness were recorded fortnightly by using HACH kit, produced by HACH, USA (Model FF-2). It was a ten parameters kit, which allows measurement of 10 critical fresh water parameters using accurate Digital Titrator and continuous color-disc colorimetry.

3.6 Culture procedure

3.6.1 Rearing in the hatchery and nursery

The larvae were reared in the hatchery up to 45 days. In the hatchery phase the larvae were fed with co-feeding of *Artemia* nauplii and custard feed (composed of milk 30 ppm/ml, egg 1 piece, corn flour 15 g, cod liver oil 0.1 g, chopped and smashed shrimp 20 g, vitamin premix 2 g) 5-6 times in a day.

After rearing in the hatchery, the post larvae (PL) were stocked separately in the nursery pond and were reared at the stocking density of 20 PL/m^2 for up to juvenile. During nursery rearing the PL was fed with Mega starter feed (Code 400, SPECTRA HEXA FEED LTD.) (36 % protein) at the rate of 20-10 % of the body weight and 3-4 times in a day.

3.6.1Grow-out culture

After nursingin the hatchery the PL was stocked in grow out pond at three different stocking densities according to the experimental design. At the beginning of the grow-out phase the dried pond bottom was treated with Super PS and after stocking it was used once in a week together with Zymetin (four times in a week). During grow-out phase, the animals were fed with Mega grower (Code 405, SPECTRA HEXA FEED LTD.) (32 % protein) at the rate of 10-3 % of the *body weight. Feed was spreaded on the water column three times* in a day at 30% (6.00 am), 30% (12.00 pm) and 40% (6.00pm) of the total feed allocated for the prawn.

3.7 Sampling

Sampling was done at every 15 days interval. Forty individuals were collected randomly at every sampling using cast net from each pond. The prawns were then kept in hapa for a short time. The length of the samples was measured using scale and the weight was measured by an electric balance with 0.001 g accuracy. At the end of the experiment, all the ponds were dewatered and all the prawn was harvested completely. Number of male, female and number of BC, OC and SM were counted.

3.8 Sexing

At initial stage, the determination of sex of prawns is very difficult. Adult males and females are easily distinguishable. Adult males are larger than the females. The second pair of walking legs or claws and the head of malesis larger than those of the females. The base of the last pair of walking legs of males is expanded inward to form a flap that covers the opening through which sperm are released. The males' walking legs are close together in nearly parallel lines, with little open space between them. A wide gap exists between the last pair of walking legs in females and they have a genital opening at the base of the third pair of walking legs. There are also differences in color between male and female. Adult females are normally yellowish. Males show blue, orange and translucent color. By observing these features, sexing was done after maturation of prawns.

3.9 Data analysis

Collected data was analyzed by Microsoft Excel and SPSS 16.0 software to make the graphical and tabular presentation of data.

Chapter

Four

RESULTS

Chapter: 4

Result:

Effects of a control dose of two probiotics were seen on the production of galda stocked in three separate densities in ponds. The probiotics were Zymetin (a feed probiotics) and Super SP (a soil probiotics) and the stocking densities were 2 juveniles/m^2 (T_1) 3 juveniles/m^2 (T_2) and 4 juveniles/m^2 (T_3). After a culture period of five months, highest production was obtained from the T_2. The specific growth rate was found higher in the T_2, however, survival rate was higher in T_1. Formation of different morphotypes in males undermines total production of galda in culture ponds; of the three morphotypes that are principally graded *viz.*, blue claw males (BC), orange claw male (OC) and small male (SM), BCs are the biggest and while the OCs are grown to medium sized range, the SMs are of the smallest sizes. It was thus interesting to see, together with the effects of probiotics, the impacts of sex ratios in the stock densities on the formation of different morphotypes and with consequent total production obtained from the different treatments. Sex ratios were noted during sampling for growth and finally recorded during total harvest and seen to be 1.02:1; 1:1.13 and 1.78:1 in the three treatments respectively and formation of BC type was found highest at 64% in T_2 and so also highest production 1158.42kg/ha was obtained from same the treatment; it is to be noted that the treatment was having least number of males than in the other two treatments.

Table (4.1) below shows the sex ratios in different treatments during sampling for growth study in galda. Sampling for growth was done in every three weeks or so. Sex ratio in the sampled

Date of sampling	Treatments					
	T1		T2		T3	
	Male %	Female %	Male %	Female %	Male %	Female %
25.09. 14	67	33	61	39	74	26
18.10.14	51	49	60	40	62	38
07.11.14	41	59	46	54	70	30
28.11.14	57	43	58	42	63	31
	Final Harvest					
Date	T1		T2		T3	
	Male %	Female %	Male %	Female%	Male %	Female%
17.12.14	5	48	47	53	64	36

Table 4.1: Sex ratios in different treatments during sampling.

galda in different dates did not have a regular trend, except in T_3 where percentage of male was recorded higher in all the sampling dates. The figure 4.1 below shows the percentages of male and female obtained in the three treatments at final harvest of the fishes. The ratios of male and female stood at 1.08:1 in T_1, 1:1.13 in T_2 and 1.78:1 in T_3, where the stoking densities were 2 juveniles/ m^2, 3, juveniles/ m^2 and 4juveniles/ m^2 respectively. The figure 4.2 shows the percentages of BC, OC and SM males in the three treatments. The highest total production (1158.42kg/ha) was obtained from T_2 with the highest BC male formation (64%). The next higher BC males were in T_1 (57%), followed by T_3 (25%). Difference between T_1 and T_3 and T_2 and T_3 were significant. ($p < .05$).The difference between T_1 and T_2 was not significant (p .> .05) with respect to stoking densities.

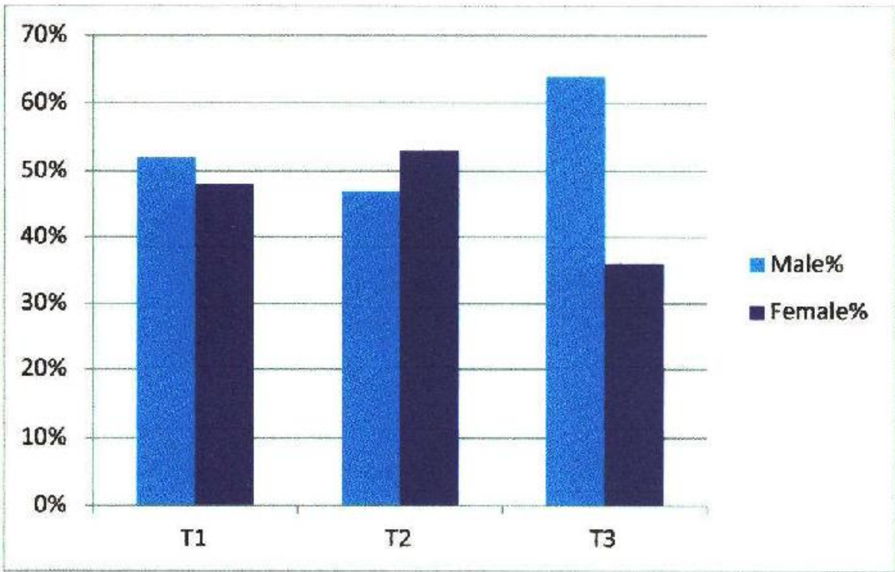


Fig: 4.1 Percentages of male and female in different stocking densities

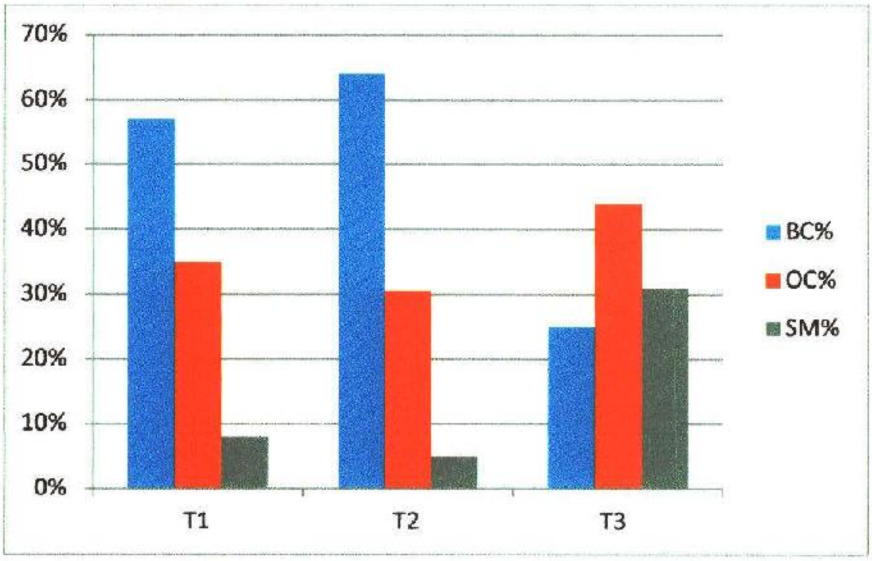


Fig: 4.2 Proportions of different male morphotypes in different stocking densities

The highest specific growth rate (SGR) 2.17 was obtained in T_2. In T_1 and T_3 the SGR were found at 2.04 and 1.89 respectively (Fig: 4.3), evidently SGR decreases at high stocking densities. In this experiment, same doses of Zymetin and Super PS (5 g Zymetin with 20 g Mutagen per kg feed in every four days in a week and 15 liter Super PS with 7.5 kg $day^{1}week^{-1}$) were applied in each treatment. The effects of these probiotics doses had differed in different densities of the treatment. The stock density 3 juveniles/m^2 had resulted highest formation of blue claw males (64%) with consequent highest total production (1158.42kg/ha).

Fig: 4.3. Effect of probiotics and stocking densities on Specific Growth Rate (SGR)

Different productions were achieved from the different treatments. Monthly productions of prawns from the three treatments are shown in figure 4.4. After final harvest, the highest production (1158.42kg/ha) was achieved from T_2. The lowest production (667.87kg/ha) was obtained from lowest stocking density T_1; the production obtained from T_3 was 787.18 kg/ha.

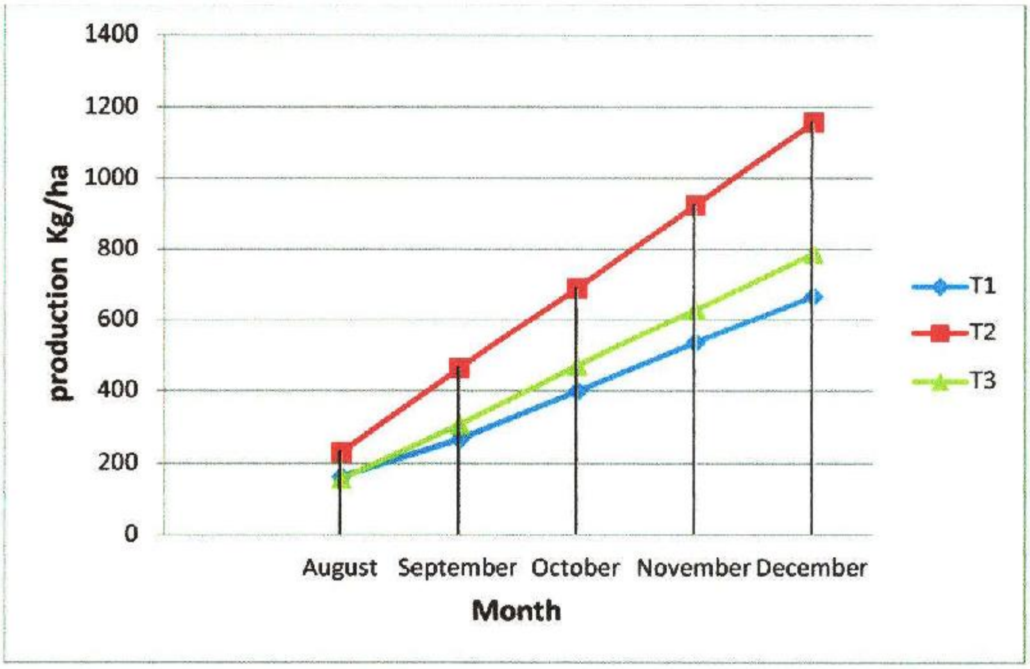


Fig : 4.4 Monthly productions of prawns in different stocking densities.

Probiotics and stocking densities also had affected the survival rates of prawns. A negative relation was found between stocking density and survival rate. With the increase of stocking densities survival rate was decreased. The survival rates in the treatments T_1, T_2 and T_3 were found to be 88.43%, 82.35% and &71.38% respectively (Fig: 4. 5).

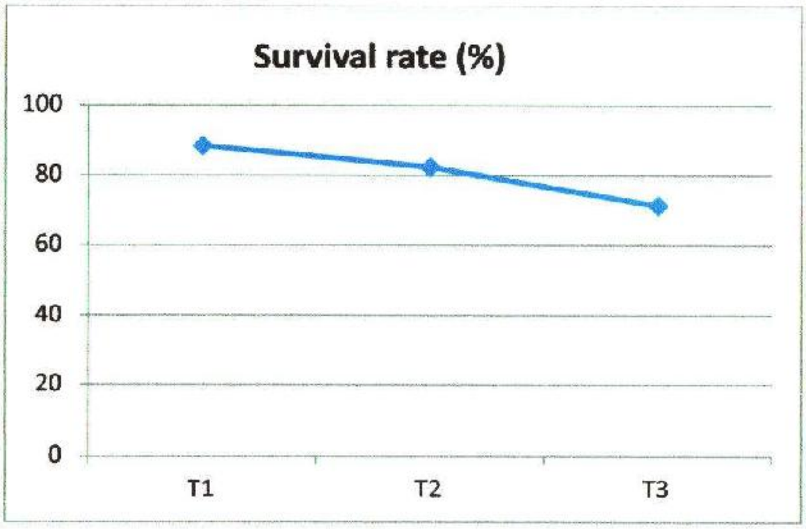


Fig: 4.5 Survival rates of prawn in different stocking densities.

Table 4.2 shows different water quality parameters of obtained from the three treatments. There was no significant difference in different parameters of water quality. These parameters did not affect the growth, formation of male morphotypes and production of prawns in this experiment.

Table: 4.2 Water quality parameters in different stocking densities.

Water quality Parameters	**Treatments**		
	T_1	**T_2**	**T_3**
	Mean±SD	Mean±SD	Mean±SD
Temperature (°C)	28.31±4.53	28.21±4.48	28.37±4.34
DO (mg/L)	4.99±0.67	5.02±0.70	5.03±0.67
pH	7.58±0.17	7.62±0.17	7.66±0.18
Salinity (ppt)	0.93±0.62	0.96±0.61	1.03±0.70
Alkalinity ($CaCO_3$ mg/L)	115.22±14.6	118.78±21.43	113.67±9.01
Hardness ($CaCO_3$mg/L)	176.3±43.7	178.8±34.2	177.4±42.8

Chapter

Five

DISCUSSION

Chapter: 5

Discussion

In many species male and female have differential growth rates and thus sexes have implications on total production and that lead to the development of mono-sex culture, either male or female for aquaculture production increase. Again, in many species, because of male competition in the culture systems, sex ratio in culture population is often a determinant of production profitability; in galda culture there is the usual formation of three conspicuous morphotypes in male. The bigger males are with blue claws, however, the orange claw and SM males respectively are of medium and smaller sized and comparative proportions of these morphotypes in the culture stock undermine the total harvest. In the present study the impact of sex ratios was analyzed on the formation of the three morphotypes in the three stocking densities employed. The stocking densities were 2 juveniles/m^2 (T_1), 3 juveniles/m^2 (T_2) and 4 juveniles/m^2 (T_3). The sex ratios at harvest were obtained to be 1.08:1, 1:1.13 and 1.78:1 in the three stocking densities respectively. The proportions of the bigger - blue claw males were 64%, 87% and 25% in the three treatments respectively with the total production being obtained was the highest in treatment 2, evidently suggesting that under probiotics application, sex ratio nearing 1:1 might be more beneficial to the formation of blue claw male in the culture system.

Stocking density can affect the proportion of male and female in the culture system. In this experiment, the sex ratio was not significantly different at low densities ($T_1$and T_2). But the number of males increased and females decreased at high density (T_3). Ranjeet and Kurup (2002) found dissimilar sex ratios at four different densities. Unlike what is obtained in the present study the number of females was found to increase with the increase of stocking rate in polder culture system. Spent females were proportionately higher in low density polders, while the undersized berried females dominated in high density polder. This may be because of the fact that under lower densities the females had a faster morphological progression and maturity which results in the appearance of larger spent females in the final harvest. In case of *Macrobrachium amazonicum*, sexually mature females were also affected by density-dependent factors. The number of spent and berried females significantly decreased at high density of stocking (80pl/m^2). (Patricia *et al.*2007).

stocking density ($80pl/m^2$) and highest production was achieved from that treatment (Patricia *et.al)* (2007). In present study of culture in pond the reason for obtaining lesser production from T_3 could be ascertained to the male competition in sex ratio 1.78:1 male: female leading to the formation of higher percentages of OC and MS males in the treatment.

The relation between stocking density and survival rate was found to be negative; at the lowest density survival rate was highest and with the increase of stocking densities, survival rate was decreased. Similar effect of stocking densities on survival rate of cultured species was found in previous related works (Nabil *et al.* 2013, Nagarathinam *et al.* 2000)). It was mentioned before that at high density the agonistic behavior, competition for space and food, mating etc. is responsible for poor growth rate which ultimately reduce the capacity of survive for species. An exceptional result was found in case of *M. amazonicum* where high density did not significantly affect the survival rate of the species (Patricia *et.al.* (2007).

Chapter

Six

CONCLUSION

Chapter: 6

Conclusion

In our country, very often farmers have to face profit loss in freshwater prawn culture due to the influences of heterogeneous individual growth, especially at high stocking densities. So, to get better production, it is necessary to have large proportion of larger BC and OC males rather than SM. The relative proportion of larger males in the final population profoundly influences the economic viability of prawn farming. Stocking density and the developing sex ratio in the stocked galda is found as an important factor affecting the proportions of different male morphotypes, growth performances, production and also survival rate. Probiotics as growth promoter might influence the fast transformation of different stages of male morphotypes. Accordingly it is suggested that, stocking density of 3 juveniles/m^2 with Zymetin (5 g Zymetin with 20 g Mutagen per kg feed) and Super PS (15 liter Super PS with 7.5 kg sand/ha/week) could be proved useful to get best output in galda culture.

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Chapter: 7

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Photo gallery

Fig 1 : A few specimens of *Macrobrachium rosenbergii*

Fig 2 : Growth sampling of *M.rosenbergii*

Fig 3: A single species has been sexed

Fig 4 : Sample specimens of *M.rosenbergii*