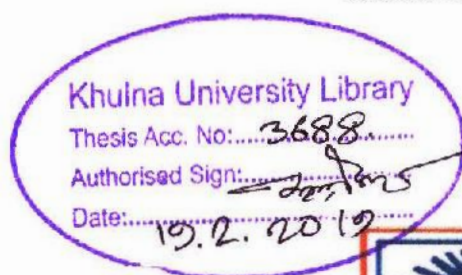


**Effect of Micro and Macro Nutrients Enrich Fertilizer on the Growth
Performance of Prawn (*Macrobrachium rosenbergii*), Rohu (*Labeo rohita*)
and Mola (*Amblypharyngodon mola*) in a Polyculture System**

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Student ID: MS-170606

Session: 2016-17



Fisheries and Marine Resource Technology Discipline

Khulna University

Khulna-9208, Bangladesh

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A Thesis By

Md. Mostafizur Rahman

Roll No: MS-170606

Session: 2016-17

The Thesis submitted to the Fisheries and Marine Resource Technology Discipline
in partial fulfillment of the requirements for the degree of

Master of Science in

Fish Processing and Quality Control

Khulna University

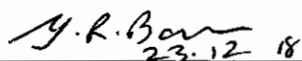
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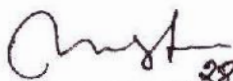
Khulna-9208, Bangladesh

DECLARATION

Effect of Micro and Macro Nutrients enrich fertilizers on the Growth Performance of Prawn (*Macrobrachium rosenbergii*), Rohu (*Labeo rohita*) and Mola (*Amblypharyngodon mola*) in a Polyculture 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.

Candidate


28.11.2018

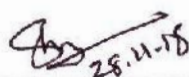
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November, 2018

DEDICATED TO.....

My Beloved Parents

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At the outset, all of my admiration goes to the supreme creator and ruler of the universe, Almighty Allah whose mercy keeps me alive and whose boundless blessings enabled me to successfully complete the thesis work for the degree of Master of Science in Fisheries and Marine Resource Technology from Khulna University under the Fisheries and Marine Resource Technology Discipline.

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Md. Mostafizur Rahman

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Abstract

A six month's experiment was carried out in the earthen ponds to evaluate the effect of micro and macro nutrients on the growth performance of major carps Rohu (*Labeo rohita*), prawn (*Macrobrachium rosenbergii*) and mola (*Amblypharyngodon mola*) in a polyculture system. The trial was conducted into two different treatments and one control each with three replications. In Treatment-1, macro nutrients like urea, TSP and molasses-yeast mixture were applied at the rate of 2.45, 2.45, and 3.09 ($\text{gm}^{-2}\text{week}^{-1}$), whereas in treatment-2 micro nutrients composition mixer applied at the rate of 4.5 $\text{g m}^{-2}\text{week}^{-1}$ while no micro and macro nutrients were applied in control. Nine water quality parameters (water temperature, pH, salinity, DO, alkalinity, hardness, nitrate, nitrite and ammonia) were measured from different treatments with comparing control unit by using standard and instrumental methods. The mean value of hardness showed significant difference while other water quality parameters had no significant differences among control and two treatments when one-way ANOVA was performed. The average final mean individual weights of prawn (*M. rosenbergii*), rohu (*L. rohita*) and mola (*A. mola*) in Treatment-1 were 24.35, 190.6, 3.67g and in Treatment-2 were 24.7, 182.2, 3.73g while 22.1, 141.1, 3.36g were in control. The final harvest weights of mola (*A. mola*) in Treatment-1 and Treatment-2 was more or less similar as control. The survival rates of prawn (*M. rosenbergii*), and rohu (*L. rohita*) were 73.33, 89.58 and 86.25%, in control 78, 74 and 74% and Treatment-1 while mola had 10.67, 15.33 and 15.33% survival rate in T-1, T-2 and control respectively. Perhaps mola (*A. mola*) fishes were consumed by prawn (*M. rosenbergii*). There was no significant difference in the survival rates of prawn (*M. rosenbergii*), rohu (*L. rohita*) and mola (*A. mola*) between control and treatments. Production after six months of culture was 351.8, 461.5 and 445.3 kg/ha in control, treatment-1 and treatment-2 respectively. Significant difference was found in the

production of prawn (*M. rosenbergii*), rohu (*L. rohita*) and mola (*A. mola*) among control and two treatments. fertilizers indirectly played a major role by increasing production of fishes in T-1 and T-2 than control. Use of macro-nutrients enriched Treatment-1 gave better overall production of fishes comparing to the Treatment-2 with micro-nutrients based commercial fertilizers. However including mola could to maximize the production of prawn and rohu in fertilizer included polyculture system though mola production may be affected by the other fishes. Therefore, inclusion of traditional inorganic fertilizers with the recommended doses of this study might be helpful for carp-prawn polyculture to meet economical and nutritional benefits.

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Chapter.....1

Introduction

1. Introduction

1.1 Background of the study

Fisheries sector in Bangladesh is very potential for the food safety and economic development of the nation. The country is an agro-based country and among different sub sectors of agriculture, fisheries sector plays an important role by contributing 3.97% of GDP which is about 23.56% of total agricultural earning and about 5.65% of the export earnings (DOF, 2017) and fish is also an important component of human diet, as a rich source of animal protein. It has huge potential to resolve the threatening problem of malnutrition (Gerking, 1966). It is one of the best and abundantly found aquatic organisms in the world with superior nutritional value. Bangladesh has been endowed with vast expanse of both marine and inland fisheries resources which possess great development potential and can support a rich variety of fish of nutritional significance and economic value. It is widely recommended that biological productivity in aquaculture ponds is limited by nutrients. Appropriate application of fertilizers has become a management protocol in aquaculture. It compensates deficiency of specific nutrients and enhances the biological productivity by autotrophic and heterotrophic process (Debeljak *et al.*, 1990). Polyculture is now the most common practice of carp culture and several species combinations and stocking rate have been developed (Hoq *et al.*, 1996). These combinations are not always considered to obtain the maximum biomass of fish from a unit area, but combining different kinds of species act as subsidiary helps to utilize all parts of the food resources that may be wasted otherwise (Tripathi, 1983). Exotic fishes (mainly carps), prawn and mola are adopted in our country for culture to obtain high production within a very short time (Akand, 1986). Fish production can be increased significantly with proper application of fertilizers in carp polyculture system. Optimizing production in pond fish culture by the use of proper fertilizers is an important task. Fertilizer is

helpful for the increase of natural food of fish i.e. plankton, benthos and periphyton. Plankton is the basic food of all the organisms living in the water. Fishes and other aquatic organisms depend on this basic food directly and indirectly. Extensive work on water quality and pond fertilization has been conducted elsewhere (Boyd, 1982). Pond fertilization enhances the growth of primary producers, which are consumed by fish, ultimately augmenting the fish crop. The application of inorganic fertilizer which contains micro and macro nutrients with a wide N: P ratio is beneficial because the nitrogen from the chemical fertilizer stimulates microbial degradation of the manure. Phosphorus and other nutrients such as copper, zinc, iron, manganese etc. in the manure are released more rapidly to the water and the rate of accumulation of organic residue in the pond bottom is reduced (Chakrabarty *et al* 1976). The addition of nitrogen and phosphorus to fish ponds stimulates pond productivity through autotrophic and heterotrophic pathways (Green *et al.* 1989). Nitrogen and phosphorus applied to ponds are assimilated directly by phytoplankton, increasing primary productivity (Boyd, 1976). Animal manures, besides containing nitrogen and phosphorus, stimulate heterotrophic production, thus increasing production in ponds (Schroeder, 1980). Although a number of studies have been conducted on the effects of inorganic fertilizers in carp polyculture (Wahab *et al.* 1994), studies on the evaluation of the effectiveness of one nutrient source versus another is difficult, standardization of the rates of nitrogen and phosphorus additions permits such evaluations. Both over and under fertilization may cause adverse effects on fish production, water quality and economic returns. Therefore it is necessary to evaluate fertilization regimes and recommend appropriate fertilization strategies to farmers in order to maximize fish production, maintain good water quality, reduce environmental bad impact and maximize economic returns. The concept of Prawn (*M. rosenbergii*), Rohu (*L. rohita*) and Mola (*A. mola*) polyculture rests on the idea that when

compatible species of different feeding habits are cultured in the same ponds, the maximum utilization of all the fish food of different column of the ponds is established. The health status also affects the micronutrient and macronutrient content of fish tissues. Excessive levels of zinc and magnesium are noted in bacterial and viral diseases and high hepatocyte activity is high (Grag and Bhatnagar, 2000). Macronutrient and micronutrient levels in tissues should be monitored to support early detection of pathological changes (Brucka-Jastrzębska *et al.* 2010). The poly culture is particularly ideal in developing countries due to certain options such as management and investments. The lower levels management strategies in traditional system involve the use of fertilizers to encourage natural productivity of planktons and to improve the levels of dissolved oxygen. Application of macro and micro nutrients enrich fertilizers in fish ponds results higher fish production than from natural unfertilized systems (Green *et al.* 1992). Therefore, the potential of addition of mola (*A. mola*), rohu (*L. rohita*) and prawn (*M. rosenbergii*) in polyculture with fertilizer needs investigation.

Generally, most of the farmers follow traditional culture system because of high feed cost (about 50-60% of total cost) which provides low production and economic return. The macro and micro nutrient enrich fertilizer can be an effective tool to enhance the production of prawn and fish in homestead pond. In addition, mola (*A. mola*) can provide sufficient protein vitamin minerals for farmers and poor community. Therefore, the present study was conducted to know the effects of different kinds of macro and micro nutrients enrich fertilizers on the growth of fish Prawn, ruhu and Mola in a polyculture system along with some limnological parameters.

1.2 Objectives of the study

- To observe the effect of macro nutrients enrich fertilizer on the growth of Prawn (*Macrobrachium rosenbergii*), Rohu (*Labeo rohita*) and Mola (*Amblypharyngodon mola*) in a polyculture system.
- To observe the effect of micro nutrients enrich fertilizer on the growth of Prawn (*M. rosenbergii*), Rohu (*L. rohita*) and Mola (*A. mola*) in a polyculture system.
- To compare the effect of macro and micro nutrients on the growth of Prawn (*M. rosenbergii*), Rohu (*L. rohita*) and Mola (*A. mola*) in a polyculture system.

Chapter.....2

Review of literature

2. Review of literature

2.1 Present status of world aquaculture

The State of World Fisheries and aquaculture 2018 highlights the critical importance of fisheries and aquaculture for the food, nutrition and employment of millions of people, many of whom struggle to maintain reasonable livelihoods. The rapidly growing human population is creating an increase in the demand for fish worldwide (Tidwell & Allan, 2001). To establish food safety for increasing population (7.3 billion in 2014), UN member states adopted the 2030 agenda for sustainable development. It sets aims for the contribution and conduct of fisheries and aquaculture towards food security and nutrition in the use of natural resources so as to ensure sustainable development in economic, social and environmental terms (FAO, 2017). Fish accounted for about 17 percent of the global population's intake of animal protein and 6.7 percent of all protein consumed (FAO, 2013). Moreover, fish provided more than 3.1 billion people with almost 20 percent of their average per capita intake of animal protein. In 2015, global aquaculture production reached 106 million tons of aquatic animals and 29.4 million tons of aquatic plants, growing at an average annual rate of 6.6 percent since 1995 (FAO, 2017).

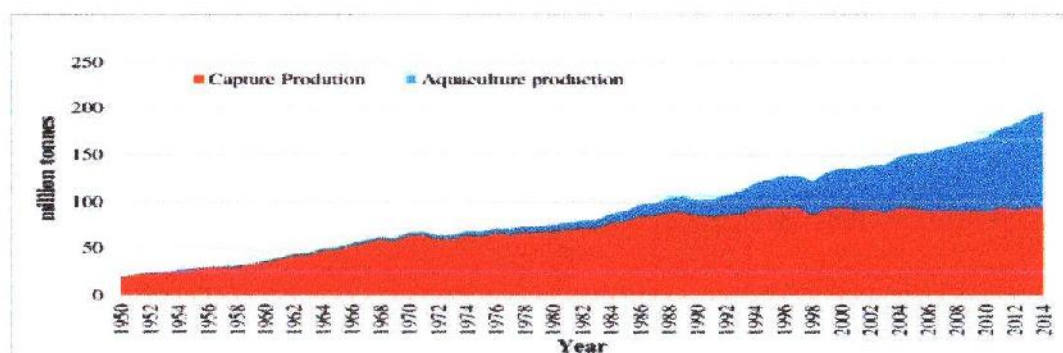


Fig. 01: World aquaculture production

Within 15 years in the new millennium, the aquaculture's contribution to the world production of aquatic animals (captured and farmed combined) has increased from 25.7% in 2000 to 45.3% in 2015 (FAO, 2017). With almost all farmed aquatic animals destined for human consumption, aquaculture supplied 10.42 kg of food fish for human consumption on world average in 2015, a level further up by 0.28 kg from 10.14 kg in 2014 (FAO, 2017). Due to over exploitation, destruction of habitat, pollution and many other causes wild fish stocks are decreasing day by day. Under these considerations, aquaculture is an effective way to ensure food security for millions of people.

Bangladesh is endowed with vast water area with a good diversity of fisheries resources. A large number of people of the country depends on fish or other fisheries resources as their livelihood since immemorial time. These sectors have also contribution on protein supply, solution of unemployment problem, earning foreign currencies and socio-economic development of Bangladesh. It contributes 3.61% to national GDP and around one-fourth (24.41 percent) to the agricultural GDP. More than 11% of total population of Bangladesh is engaged with this sector on full time and part time basis for their livelihoods (DoF, 2017). Bangladesh has achieved self-sufficiency in fish production with an annual output of 41.34 lakh MT against a demand of 40.50 lakh MT in 2016-17. The country earned about BDT 42876.40 million by exporting almost 68.31 thousand MT of fish and fisheries products in 50 countries (DoF, 2017). Bangladesh ranked fifth in world aquaculture production (FAO, 2016). In 1983-84, the contribution of inland capture and culture fisheries to total fish production were 62.59 % and 15.53 percent respectively; whereas in 2016-17, inland capture fisheries contributes only 28.14% and inland culture fisheries contributes 56.44 percent to total fish production (DoF, 2017). During the last eight years, the

aquaculture production became more than double (10.63 lakh MT in 2008-09 to 23.33 lakh MT in 2016-17). Climatic condition of Bangladesh is suitable for culture of giant fresh water prawn (*M. rosenbergii*). Among the twenty four freshwater prawns, *Macrobrachium rosenbergii* has significant aquaculture potential for its commercial value throughout the world (Akand and Hasan, 1992). The poly culture sectors have gradually grown over the last three decades and provide direct and indirect employment opportunity for around 1.5 million people (Ahmed et al., 2008). Total area of prawn and shrimp farm was 272717 ha and production was 246406 MT in 2016-17 which has contributed 5.96 % of country's total production as well as nearly double in terms of volume compared to total production in 2002-03 of 1.60 lakh MT (DoF, 2017). In 2016-17, Bangladesh exported 6627.37 MT freshwater prawn with a value of about 820.28 crore BDT (DoF, 2017). Despite the great potentiality of freshwater prawn culture in Bangladesh, there have been some concerns in recent years for sustainable prawn farming which provide less stress on environment as well as economically profitable.

2.2 Present status of aquaculture in Bangladesh

A total of 260 fish and 24 prawn species are known to inhabit in the freshwaters of Bangladesh with 12 exotic fish species have been recorded in the Bangladesh (Rahman, 2005). At present, major carps species such as *Catla catla*, *Labeo rohita*, *Cirrhinus mrigala* and *Labeo calbasu* along with exotic carp, such as silver carp (*Hypophthalmichthys molitrix*); grass carp (*Ctenopharyngodon idellus*) and common carp (*Cyprinus carpio*) are the available carp species in the market. There are about 40–50 small indigenous fish species which grow to a maximum length of 25 cm (Felts et al. 1996). Some more commonly found species of this variety include

Puntiusticto, *Amblypharyngodon mola*, *Colisalalius*, *Anabas testudineus* and *Glossogobius giuris*.

The IUCN (2000) reported that many of the small indigenous fish are now endangered or critically endangered. Among them, only carps species contribute 33.57% in total annual production (freshwater and marine water) followed by pangas (11.404%), and tilapia (8.44%) (FRSS, 2016). However, the inland water resources of Bangladesh offer major potential for the development of freshwater capture and culture fisheries (Hossain *et al.* 2014). The catch from the inland capture fishery is noteworthy, especially for rural areas in terms of fish supply and employment. Fishery products that are harvested from inland waters in minor amounts are other catfish (Boal, Air, Silon, Rita), snakehead (Shol, Gazar, Taki), other carp (Kalibaus, Bata, Ghania), and live fish (Koi, Singhi, Magur). Species-wise annual fish production in inland and marine fisheries in bangladesh for 2014–2015 are presented in Fig-02.

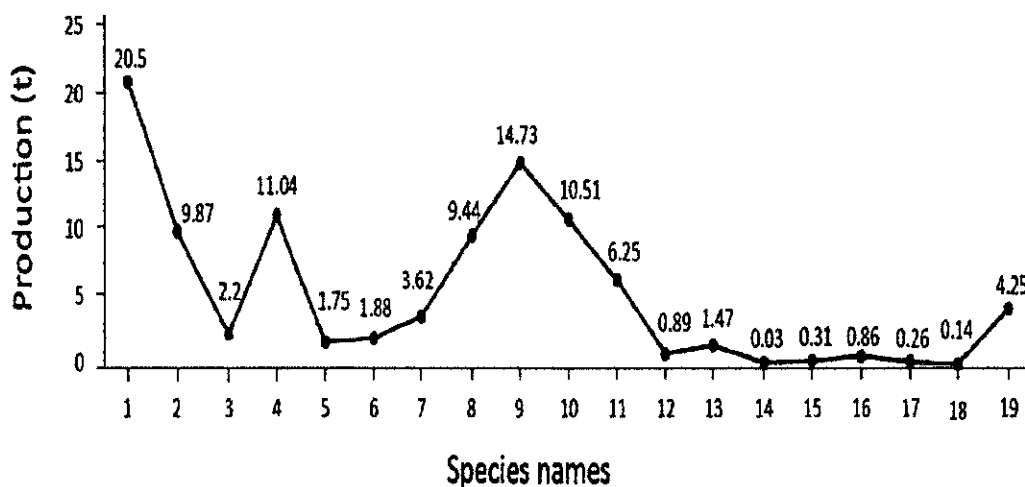


Fig. 02: Species-wise annual fish production in inland and marine fisheries 2014–2015 in Bangladesh

(1. Major carp, 2. Exotic carp, 3. Other carp, 4. Pangas, 5. Other cat fish, 6. Snake head, 7. Live fish, 8. Tilapia, 9. Other inland fish, 10. Hilsha/Illish, 11. Shrimp/Prawn, 12. Sardine, 13. Bombay duck, 14. Indian salmon, 15. Pomfret, 16. Jew fish, 17. Sea cat fish, 18. Shark/Skate/Ray, 19. Other Marine fish)

2.3 Use of micro and macro nutrients in aquaculture and its impact

All living organisms are composed of organic and inorganic compounds, which are equally important for growth and development. The quantitative and qualitative proportions of those compounds depend on a fish species, fish biology and environmental conditions. Subject to their concentrations in an organism, mineral nutrients are generally classified into two major groups: micro minerals – below 50 mg kg⁻¹ and macro minerals – above 50 mg kg⁻¹. Fish can ingest micro and macro nutrients from food or directly from water. Absorption processes are determined by various factors, mostly nutrients concentrations. The nutritional requirements of farmed fish are met by a balanced diet that contains protein, fat, carbohydrates, nutrients , vitamins which is specifically formulated for fish of a given age and species.

Micro and macro nutrients deficiencies may go unnoticed due to an absence of clinical symptoms in fish. Nutrition's deficiencies can lead to health problems, lower feed conversion efficiency and lower weight gains. Nutrition's concentrations in water should be determined before starting any supplementation regimes in fish farms. Marine fish live in a hypertonic environment, where they are exposed to constant risk of dehydration due to loss of water through gills. They have to compensate for that loss by regularly consuming small quantities of water, equivalent to 50% of their body weight, on a daily basis (NRC 1983).

Most marine fish receive their daily dose of micronutrients, such as calcium, copper, iron, magnesium, potassium, sodium, selenium and zinc from water rather than from food (Davis, Gatlin 1996). Microelements are absorbed from water, which is ingested through the mouth and taken through the gills, skin and fins. Freshwater fish are at constant risk of over hydration and salt loss in a hypotonic environment. They drink very small amounts of water to compensate for the minerals lost with urine and receive nutrients from water. In contrast, to freshwater fish, marine fish have a lower demand for dietary minerals as they absorb most of them from ambient Water (Cowey 1977). The required dietary supplementation with macronutrients and micronutrients is very difficult to determine and the amount of nutrients absorbed by fish from water is equally difficult to measure. The interactions between elements should also be described. Due to mutual interactions between ions and chemical compounds, the biological effect of an element is determined by the presence of other ions or chemical substances. Two types of reactions are generally reported: synergism (increased effect) and antagonism (decreased effect). Many authors have observed that phosphates can limit the absorption of most micronutrients by forming insoluble phosphates. Most mineral nutrients reach the heart, liver, kidneys and brain within minutes after absorption. They are then transported to muscles, skin and adipose tissue. This process is slower, and a mineral balance in bodily tissues is achieved within 30 minutes to several hours. Mineral nutrients are distributed throughout the body mainly by the circulatory system. They are carried with the bloodstream to organs and tissues, and excess nutrients are excreted at rates that are determined by the blood flow through bodily tissues.

Physiological blood parameters are highly differentiated in fish, and they are determined by individual variation, age, type of farm, diet and season. They can fluctuate widely in healthy fish, which is why physiological reference values are much more difficult to determine in fish than in endothermic animals (Allen, 1993). The mineral content of tissues may vary, and mineral concentrations are a species-specific trait. The health status also affects the micronutrient and macronutrient content of fish tissues. Excessive levels of zinc and magnesium are noted in bacterial and viral diseases and when hepatocyte activity is high. Macronutrient and macronutrient levels in tissues should be monitored to support early detection of pathological changes. The concentrations of mineral nutrients change rapidly and precede behavioral changes and other symptoms of disease (Brucka-Jastrzębska et al. 2009).

2.3.1 Macronutrients

2.3.1.1 Calcium (Ca)

In general, Ca and P co-exist together in bodies of animals, and a deficiency of one nutrient limits the availability of both minerals. 99% of calcium and 80% of phosphorus are stored in bones, teeth and scales. The remaining calcium is widely distributed among various organs and tissues. Calcium has the abundant functions in the body of a fish. It is closely related to the development and maintenance of the skeletal system and participates in several physiological processes, including the maintenance of acid–alkaline equilibrium, osmoregulation, muscle contraction, bone mineralization, blood clotting, nervous signal transmission, maintenance of cell membrane integrity, and activation of several important enzymes. This macronutrient is readily derived from the water and occurs in adequate amounts in most diets consumed by fish. Regulation of Ca influx and efflux occurs at the gills, fins, and oral epithelia. The endocrine

control of Ca metabolism in fish is also regulated by hyper- and hypocalcemic hormones. Teleosts possess two hormones with hypocalcemic action: calcitonin, secreted by the ultimobranchial gland, and stanniocalcin (STC), secreted by the corpuscles of Stannius. Symptoms of Ca deficiency were observed only in experimental conditions, and they are rarely detected in natural habitats, where Ca is absorbed from water and food. Fish may be deficient in Ca if CaCO_3 concentrations in water drop below 5 mg l^{-1} . Kalantarian et al. (2013) compared weight gains in juvenile rainbow trout administered feed with a different Ca content: 0.95%, 1.21%, 1.41% and 1.61%. There were no significant difference observed between the groups, which suggests that main source of Ca is water.

2.3.1.2 Phosphorus (P)

Phosphorus is a component of various organic phosphates, such as nucleotides, phospholipids, coenzymes, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA). Inorganic phosphates also serve as important buffers to maintain normal pH of intra- and extracellular fluids. In contrast, to calcium, the main source of P is diet (Davis and Gatlin 1996). The phosphorus requirements of rainbow trout and carp were determined in the range of 0.5 to 0.8 g 0.1 kg^{-1} of feed. The availability of phosphorus is largely influenced by its form and source. Phosphorus absorption is low in rice bran, average in fish meal and high from casein and brewer's yeast, which is due to different forms of phosphorus. The more soluble phosphate salt is, the higher the absorption. Acidic Ca, Na and K phosphates are characterized by high availability. Fish with less active gastric juices (thermophilic species such as the carp) absorb dietary P less efficiently. In general, the bioavailability of phosphorus is positively correlated with the solubility of the mineral in water. For rainbow trout, monobasic phosphates of sodium and potassium are highly

available (90 to 95%) sources of phosphorus. For common carp, the bioavailability of phosphorus from a diet is more variable, ranging from 13% (tribasic calcium phosphate) to 94% (monobasic calcium phosphate). Some nutrients affect the availability of certain minerals. For example, large intakes of iron, aluminum and magnesium may reduce the absorption of phosphorus by forming insoluble phosphates (Davis and Gatlin 1996).

2.3.1.3 Magnesium (Mg)

Magnesium is essential for maintenance of intra- and extracellular homeostasis of fish. Fish absorb magnesium supplied with water through gills or from the gastrointestinal tract. Research has demonstrated that Mg concentrations of 46 mg l⁻¹ in water are sufficient to meet the rainbow trout's demand for this nutrient. At Mg concentrations of 1 to 3 mg l⁻¹ in water, fish feed should be supplemented with magnesium at 0.025 to 0.07%. Seawater contains high levels of Mg (1350 mg l⁻¹), which is absorbed directly from water, and therefore this element does not have to be additionally supplemented in feed. The majority of nutrients, in particular those of plant origin, are a rich source of Mg and fully meet the demand for this mineral in fish (Davis, Gatlin 1996). Higher levels of magnesium in blood plasma were observed in fish from water rich of this element (Kopp *et al.* 2013). Magnesium in fish meal is characterized by low availability (Watanabe *et al.* 1988), and selected commercial feeds may not fully satisfy Mg requirements of fish. Dietary magnesium deficiency signs include poor growth, anorexia, lethargy, muscle flaccidity, convulsions, vertebral curvature, high mortality, and depressed magnesium levels in the whole body, blood serum and bone (Davis, Gatlin 1996).

2.3.1.4 Sodium, potassium, chlorine (Na, K, Cl)

Sodium, potassium and chlorine levels in water are generally sufficient to meet fish's basic nutritional requirements, but fish diets should be characterized by optimal Na, K and Cl concentrations for the optimal growth and development. In a study on juvenile rainbow trout, the highest weight gains were observed in a group whose diet was supplemented with 1.1% K. The lowest weight gains were noted in fish fed diets with the addition of 0.9% and 1.3% K (Kalantarian et al. 2013). Symptoms of potassium deficiency, such as poor appetite, tremor, tetany and death, are rarely reported. Symptoms of Na and Cl deficiency are not observed. However, at low pH, Na and Cl intake through gill epithelium is decreasing, and absorption of these elements from a diet is higher (Kopp *et al.* 2013). Dietary levels of K significantly influence the growth of fish. NaCl supplementation can also create additional benefits. NaCl supplements stimulate the Na/K pump activity in gill microsomes and facilitate adaptation to new environments. Higher chloride concentrations in water protect fish against the harmful effects of nitrates (Krupova et al. 2005).

2.3.2 Micronutrients

2.3.2.1 Iron

Iron (Fe) is a highly dispersed element in nature, and it is mobilized during rock weathering. Iron concentrations in water vary considerably due to the influence of numerous factors. Iron levels are determined at 0.06-44 $\mu\text{g l}^{-1}$ in saltwater and 10-1400 $\mu\text{g l}^{-1}$ in fresh water. Iron compounds are highly soluble in water with $\text{pH} < 7$, but they are readily oxidized and precipitated as oxides in surface water. Colloidal iron oxides play an important role in the sorption and coagulation of other colloidal substances and ions, in particular trace metals. In river water, Fe binds to other

compounds, and in saltwater with high pH. Natural water resources are a poor source of iron, and the element has to be supplied with food. Iron is essential for life, being involved in oxygen transfer, respiratory chain reactions, DNA synthesis, and immune function. Iron can be absorbed through gills, and the addition of iron sulfate has been found to stimulate growth and increase hemoglobin levels. Fe^{2+} ions are more bioavailable than Fe^{3+} ions. Reducing substances such as vitamin C increase Fe absorption, not bound to hem. There are no mechanisms that control Fe excretion, but iron absorption is strictly regulated to balance Fe levels in the body (Shi and Camus 2006). The nutritional value of waterborne iron compared to dietary iron has not been elucidated, but the gills may play a vital role in iron homeostasis at times of developmental need, for example after yolk-sac absorption and prior to feeding (Bury et al. 2003). When dietary levels of Fe are low, more iron is absorbed from water, and up to 85% of Fe can be supplied through gills (Cooper and Bury 2007). Higher levels of iron in water leads to elevated level of this element in blood plasma. This proves that ambient water can be a source of iron for fish (Kopp et al. 2013). An iron overdose can have adverse effects, including retarded growth, higher mortality, diarrhea and liver damage eg. iron levels higher than 1.380 mg kg^{-1} feed are toxic for trout.

2.3.2.2 Cobalt (Co)

Its main role is to be an intrinsic part of vitamin B12 (cobalamin). Fish are not capable of synthesizing this vitamin, therefore, dependent on bacterial production of this essential compound. Cobalt is absorbed from water through gills or ingested with food. Cobalt absorption from water with a rise in water temperature and decreases with a rise in Ca levels (Comhaire et al. 1997). A study on the carp has revealed that in fish fed diets without additional

supplementation, the highest Co concentrations are noted in the kidneys, followed by the stomach, liver and gills. At higher dietary concentrations of Co, its levels increased in the kidneys and liver, and when the recommended dietary allowance was exceeded, Co concentrations increased in the stomach, kidneys, gills and liver. The above results suggest that cobalt undergoes homeostatic regulation and that the highest dietary doses can exceed the healthy range in tissues (Murkherjee and Kaviraj 2009). Dietary Co supplementation increases weight gains, improves hematological parameters (higher red blood cell counts) and survival of hatchlings, and it stimulates protein synthesis (Watanabe *et al.* 1997). Murkherjee and Kaviraj (2009) demonstrated that supplementation of carp diets with 0.1% to 1.0% cobalt improved weight gains. Higher weight gains without additional cobalt deposition in tissues were reported at 0.1% supplementation, whereas high levels of supplementation (1%) increased cobalt accumulation in various tissues. Very high doses of dietary Co (0.1-5 g kg⁻¹ feed) can contribute to hemorrhage in the digestive tract and changes in white blood cells (Watanabe *et al.* 1997).

2.3.2.3 Zinc (Zn)

Zinc is essential due to its vital structural and catalytic function in over 300 proteins that play important roles in fish growth, reproduction, development, vision and immune function (Bury *et al.* 2003). Fish absorb Zn from water and food, but dietary Zn is more bioavailable. Absorption from water can be increased up to 50% in some cases, e.g. when a diet is deficient in this element. Also, absorption from food decreases if the water concentration of Zn is very high (Niyogi *et al.* 2007). Gills may act to supplement absorption when required. Ca and P reduce the absorption of Zn by binding it to insoluble phosphate complexes that are not absorbed from intestines. The bioavailability of zinc from feeds containing phytates and calcium phosphate is

low, and its dietary levels should be increased through supplementation (When zinc was supplemented at 20 mg kg⁻¹ feed, the highest weight gains were observed in fish fed zinc sulfate, and the lowest gains occurred in fish fed zinc chloride (Watanabe et al. 1997).

Zn is not abundantly stored in tissues because it is absorbed mainly through gills, where the highest zinc concentrations are found. Changes in tissue concentrations of Zn can be attributed to this element's affinity to the membranes of erythrocytes and serum proteins participating in its transport. Zinc is characterized by low toxicity, and its negative influence on the body is mainly due to secondary copper deficiency. Zinc absorption is determined by feed quality and interactions with other elements. Zinc is a major antagonist of Cd and Cu, and zinc absorption can also be reduced by Ca and Mg (Brucka-Jastrzębska *et al.* 2019). Excessive Zn levels decrease weight gains (in the carp, weight gains decrease when Zn concentrations exceed 300 mg kg⁻¹), hemoglobin levels, hematocrit levels and Zn concentrations (at 1000 mg kg⁻¹ in the trout) (Bury et al. 2003). Symptoms of poisoning may be observed already at very low Zn levels of 1 mg l⁻¹ water (Watanabe et al. 1997).

2.3.2.4 Manganese (Mn)

Manganese can be absorbed from water through gills, but dietary manganese is more bioavailable for fish. Its availability is limited at high concentrations of Ca and in the presence of phytates (Watanabe et al. 1997). In dietary supplements, Mn is more available when supplied in the form of manganese sulfate or manganese chloride than manganese carbonate. Higher weight gains, increased protein synthesis and decreased lipid synthesis in the liver were observed in carp whose diet was supplemented with manganese sulfate (Watanabe et al. 1997).

2.3.2.5 Copper (Cu)

In fish, the demand for copper is met mainly from dietary sources, but copper is also absorbed directly from water. When adequate amounts of Cu are supplied with feed and water, 10% of copper requirements are met through absorption across the gill epithelium. When dietary concentrations of Cu are insufficient, gill absorption is intensified to cover up to 60% of demand. When dietary Cu is overdosed, gill absorption drops below 1% (Kamunde *et al.* 2002).

The majority of feeds and water are adequate sources of Cu, but Cu requirements are largely determined by the physiological state of fish and the concentrations of Zn, Fe, Cd and Mo, which are antagonists of Cu (Watanabe *et al.* 1997). At present, Cu concentrations of 1-2 $\mu\text{g l}^{-1}$ are rarely encountered in rivers. Copper levels fluctuate subject to geological conditions and anthropogenic pollution. Copper accumulated in bottom deposits is a potent indicator of pollution caused by human activities. The discussed element can be transported to underground water resources. Excessive Cu levels in water are highly detrimental to self-cleaning mechanisms. Cu concentrations of 20 $\mu\text{g l}^{-1}$ in acidic soft water and 250 $\mu\text{g l}^{-1}$ in hard alkaline water can be toxic to trout (Hellawell 1988). An overdose of Cu retards the growth and reduces feed conversion efficiency and hematocrit levels (at 730 mg kg⁻¹ Cu in trout). Cu concentrations of 0.8-1.0 mg l⁻¹ water are toxic, but pathological symptoms appear only at very high levels of Cu supplementation (feed containing 600 mg kg⁻¹ Cu did not exert a harmful impact on rainbow trout). Adverse effects of copper are mitigated by gastric mucosa, which acts as a barrier to toxic metals (Handy 1993). Dietary levels of Cu higher than 730 mg kg⁻¹ inhibited the growth and decreased feed intake. A copper overdose can also lead to gill damage (Watanabe *et al.* 1997).



2.3.2.6 Iodine (I)

Iodine is found in all animal tissues, and sea organisms are particularly rich in this element. Iodine is easily absorbed from food, water and air. It is a mobile element, which is not accumulated in the body. Up to 80% of absorbed iodine is transported to the thyroid gland. Iodine levels in the blood of freshwater fish range from 0.5 to 2000 $\mu\text{g l}^{-1}$ (Watanabe et al. 1997). They are determined by iodine concentrations in feed, water and tissues as well as the ability of blood proteins to bind this element. Triiodothyronine (T_3) is the major thyroid hormone and an active precursor of thyroxine (T_4). Fish differ from mammals in their utilization of iodine and extrathyroidal metabolism of T_3 and T_4 (Watanabe et al. 1997). T_3 binds more strongly to blood proteins than T_4 , and its plasma levels are lower in comparison with T_4 . Both T_3 and T_4 are evacuated mainly with bile, but also through gills and kidneys (Watanabe et al. 1997). Iodine absorption is determined by physiological and dietary factors, and a secondary deficiency is not always associated with low iodine doses. Se and Fe as well as Co, Cu, Ca and Mn affect thyroid functions, decrease iodine absorption or disrupt iodine metabolism. Iodine-deficient feed and feed ingredients that promote goiter formation, such as rapeseed, are harmful to farm animals. Hyperthyroidism (goiter) induced by iodine deficiency is more frequently observed in carnivorous than herbivorous and omnivorous fish (Watanabe et al. 1997). Excessive dietary intake also leads to thyroid dysfunction. In animals, symptoms of iodine overdose include mucosal inflammation, cell necrosis and iodine allergy. In iodine intolerance, even small doses of iodine lead to skin changes and mucosal inflammation. In a laboratory experiment, rainbow trout absorbed 80% of iodine from water, 19% from feed and less than 1% from decomposition of thyroid hormones. High Ca concentrations in water and high Co intake inhibit iodine

absorption. Saltwater is more abundant in iodine, which is why saltwater fish are rarely deficient in this element. Fish feed should be supplemented with minimum 2.8 mg kg⁻¹ I (Watanabe *et al.* 1997). Iodine requirements are significantly determined by stress exposure as well as the age and physiological condition of fish.

2.3.2.7 Selenium (Se)

Fish absorb selenium from both water and feed. Selenium is ingested in much larger quantities with water through gills, and it is stored in various tissues, excluding the liver, in the inorganic form. Dietary selenium is accumulated in the organic form, but the threshold between healthy and toxic. Se concentrations are relatively narrow. Symptoms of toxicity are observed at 13-15 mg Se kg⁻¹ feed or 40-130 µg Se l⁻¹ water. In water, selenium levels generally remain below 0.1 µg l⁻¹ (Watanabe *et al.* 1997).

Polyculture is now the most common practice of carp culture and several species combinations and stocking rate have been developed (Hoq *et al.* 1996). These combinations are not always considered to obtain the maximum biomass of fish from a unit area, but two or more species act as subsidiary compatible species that will utilize parts of the food resources that may be wasted otherwise (Tripathi, 1983). Exotic fishes (mainly carps) are adopted in our country for culture to obtain high production within a very short time. A wide range of exotic carps viz. common carp, bighead carp, silver carp, grass carp, black carp etc have performed better results (Akand, 1986). Fish production can be increased with accurate application of fertilizers and supplementary feeds in carp polyculture system. The main purpose of pond fertilization is to augment 130 fertilizer effect on growth of exotic carps the production of planktons which serve as natural food of the

fishes; because fertilization stimulates both the autotrophic and heterotrophic levels of natural food which increase fish production (Grag and Bhatnagar, 2000). Fish production can be increased upto 5,000 kg/ha/year by feeding and fertilization (Ekram, 2002).

The concept of carp polyculture rests on the idea that when compatible species of different feeding habits are cultured in the same pond, the maximum utilization of all the fish food of different column of the pond is established. Though fertilization of ponds has been identified as an indispensable management practice for optimizing fish production, the farmers use three or four times higher than the standard dose. However, presently crisis of the chemical fertilizers in the country is an acute problem (Ekram, 2002). Moreover, the farmers are facing financial loss for buying excess fertilizers. So far, there has been little works to determine the optimum dose of fertilization is needed for maximum fish production in carp polyculture. Considering the above scenario, the present study was carried out to evaluate the effects of inorganic fertilizers with constant supply of supplementary feeds on the growth, survival and production of the exotic carps in polyculture system as well as to monitor the changes of physico-chemical parameters of ponds water.

Chapter.....3

Materials and method

3. Materials and Methods

3.1. Study area and period

The study was conducted from the experimental pond complex II at Fisheries and Marine Resource Technology Discipline of Khulna University, Bangladesh during July to December, 2017. All ponds were rectangular in shape with a maximum depth of 1.5 m. All the ponds were fully exposed to prevailing sunlight.

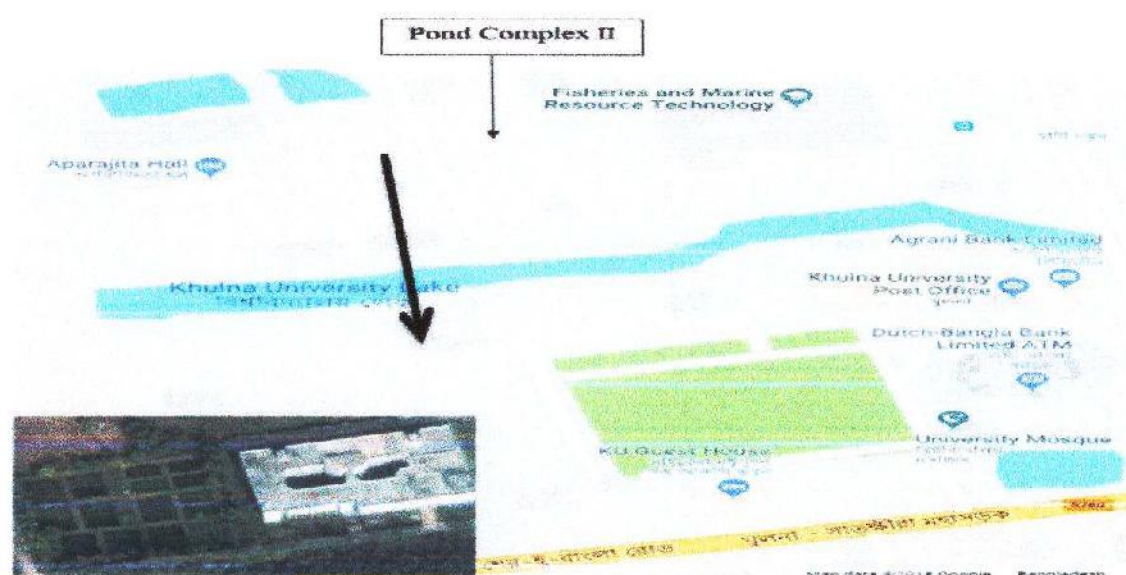


Fig. 03: Representative Map of Study Area

3.2 Experimental Design

The trial was conducted into two different treatments and one control with three replications. Stocking density of prawn (*M. rosenbergii*), rohu (*L. rohita*) and mola (*A. mola*) were same in all the treatments. The experimental design is summarized in the Table 1

Table 01: Research design

Parameter	Control	Treatment-1	Treatment-2
Area of each pond	120 - 150 m ²	120 - 150 m ²	120 - 150 m ²
Water depth	1-1.5 m	1-1.5 m	1-1.5 m
Prawns (<i>M. rosenbergii</i>)	2/m ²	2/m ²	2/m ²
Rohu (<i>L. rohita</i>)	0.1/m ²	0.1/ m ²	0.1/ m ²
Mola (<i>A. mola</i>)	2/m ²	2/m ²	2/m ²
Fertilizer	No	Traditional inorganic fertilizer	Commercial inorganic fertilizer
Dose		Urea 2.45, TSP 2.45, Molasses-yeast mixture 3.09(g/m ² /week)	Before stocking: 2kg/1000 m ³ of water During culture period: 1kg/1000m ³ /week
Composition		Urea, TSP, Molasses-yeast mixture	Dicalcium Phosphate:500000mg Cobalt:120 mg Copper:10000 mg Choline:6000 mg Folic Acid:120 mg Biotin:5mg Manganese:2000mg Selenium: 100gm Zinc:11000mg Iron:20000mg Pantothenic acid:1300mg Vit-A:300000UI, Vit-D ₃ :160000UI Vit-E: 2000mg, Vit-K ₃ : 200mg Vit-B ₁ :600mg, Vit- B ₂ :300mg Vit B ₆ :300mg
Replication	3	3	3

3.3 Pre-stocking management

At the beginning of June, ponds were renovated and cleaned of aquatic vegetation. All unwanted fishes and other aquatic organisms were eradicated by dewatering. Pond dikes were repaired using the excavated bottom soils. The weeds of embankment were also cleaned manually. Surroundings of all ponds were fenced by 1 m high nylon net to prevent the entry of fishes, snakes and other predators, and escape of fishes. Lime was applied to the pond bottom at the rate of 250 kg ha^{-1} . The ponds were filled with water after seven days of liming from adjacent deep tube well by using pump. As presented in Table 1, all ponds of Treatment 1 were fertilized with inorganic fertilizers like, urea 2.45, TSP 2.45, molasses-yeast mixture 3.09 ($\text{g m}^{-2}\text{week}^{-1}$) and micro nutrients composed mixer applied at a rate of $4.5 \text{ g m}^{-2}\text{week}^{-1}$ in treatment 2, while no fertilizers were applied in the control. After the first fertilization, the ponds were left 10 days to allow plankton growth before stocking fishes.

3.4 Stocking

3.4.1 Stocking in nursery pond

Prawn PL was purchased from a commercial hatchery in Coxes bazar during middle of May 2017 and stocked in nursery pond. While rohu fry and brood mola were purchased from a local seller in Khulna at July 2017.

3.4.2 Stocking in grow-out ponds

Prawn, rohu and mola were stocked in grow out ponds during July 2017. The average initial weight of prawn, rohu, and mola were 2.32, 94.13 and 2 g respectively in both two treatments and control as well. Before stocking, fish fry and prawn juveniles were conditioned in a hapa.

3.5 Post stocking management

All ponds were subjected to the same regime of feed and fertilizer. Feed was provided as regular basis of traditional culture system. Fish are fed daily at the rate of 5% body weight throughout the study period. Half of the required food was applied evenly over the surface of the ponds, in the morning and the rest half in the evening. Fertilization with urea, TSP and molasses-yeast mixture was done at the rate of 2.45, 2.45, 3.09 ($\text{g/m}^2/\text{week}$) respectively at every 7-day interval in T_1 to keep natural food available in the water and micro nutrients composition mixer applied at the rate of $4.5\text{g m}^{-2}\text{week}^{-1}$ in Treatment-2 While no fertilization is applied in control.

3.5.1 Water sampling and analysis

Water quality parameters like temperature, transparency, DO, pH, alkalinity, hardness, nitrate-nitrogen ($\text{NO}_3\text{-N}$), nitrite nitrogen ($\text{NO}_2\text{-N}$), and ammonia-nitrogen ($\text{NH}_3\text{-N}$) were measured fortnightly. Some of the important analytical methods in some cases with some modification were conducted in the water chemistry research laboratory of Fisheries and Marine Resources Technology (FMRT) Discipline, The water quality parameters like temperature, pH, dissolved oxygen (DO), alkalinity, hardness, ammonia (NH_3), nitrite, nitrate were recorded fortnightly.

3.5.2 Growth check

Five individuals of each species from each treatment are sampled monthly to check the growth and health condition as well as for feed adjustment.

The following parameters were used to evaluate the growth of fishes:

- a. Weight gain (g) = Average final weight (g) – average initial weight (g)
- b. Survival rate (%) = (No of fish harvested/ No of fish stocked) x 100

3.6 Harvesting of fish

Ponds were completely harvested by seine net after six months of rearing. All fishes were counted and weighed separately to assess survival rate and production.

3.7 Statistical analysis

The data collected during experiment were recorded. Data were analyzed using MS excel and the statistical package SPSS (16). One-way ANOVA test was performed to identify any significant differences among treatment means. Significance was assigned at the 5% level.

Chapter.....4

Results & Discussion

4. Results and Discussion

4.1 Water quality

Water quality parameters were analyzed to observe any considerable changes that might have occurred in response to different condition in treatments. Various physical, chemical and biological parameters of pond water were determined under control and two treatments. All parameters were present within the acceptable range for fish culture. Water quality parameters in control and treatments have been presented in Table 02.

Table 02: Mean ($\pm$ SD) values and ranges of water quality parameters

Parameters	Experimental groups		
	Treatment-1	Treatment-2	Control
Temperature (°C)	25-31	25-31	25-31
	28.50 $\pm$ 2.06 ^a	28.73 $\pm$ 2.23 ^a	28.86 $\pm$ 2.30 ^a
DO (mg/l)	6-8	6-8	6-8
	7.09 $\pm$ 0.94 ^a	7.36 $\pm$ 0.92 ^a	6.64 $\pm$ 0.92 ^a
pH	7.35-8.7	7.5-8.7	7.5-8.7
	8.32 $\pm$ 0.42 ^a	8.33 $\pm$ 0.37 ^a	8.23 $\pm$ 0.44 ^a
Alkalinity (mg/l)	200-300	200-300	200-275
	247.73 $\pm$ 32.51 ^a	245.45 $\pm$ 31.26 ^a	220.45 $\pm$ 24.54 ^a
Hardness (mg/l)	165-200	165-200	155-170
	184.55 $\pm$ 11.93 ^b	183.18 $\pm$ 14.19 ^b	164.09 $\pm$ 5.39 ^a
Nitrite (mg/l)	0.0285-0.9715	0.0235-0.1095	0.0235-0.1095
	0.08 $\pm$ 0.04 ^a	0.09 $\pm$ 0.03 ^a	0.07 $\pm$ 0.03 ^a
Nitrate (mg/l)	0.13-0.285	0.13-0.285	0.125-0.235
	0.21 $\pm$ 0.05 ^a	0.20 $\pm$ 0.05 ^a	0.17 $\pm$ 0.03 ^a
Ammonia (mg/l)	0.0415-0.138	0.0415-0.138	0.038-0.107
	0.09 $\pm$ 0.03 ^a	0.09 $\pm$ 0.03 ^a	0.07 $\pm$ 0.02 ^a

*** Figures in the same row with the same superscript are not significantly different.

4.1.1 Temperature

The water temperature of experimental ponds was found to vary from 25 to 31 °C during the experiment in control and two treatments respectively. The highest mean temperature was recorded on the September (31°C) while the lowest was recorded on the December (25°C) in both control and treatments. No significant difference ($P > 0.05$) in temperature was found among control and treatments (Kunda *et al.* 2008) reported similar findings.

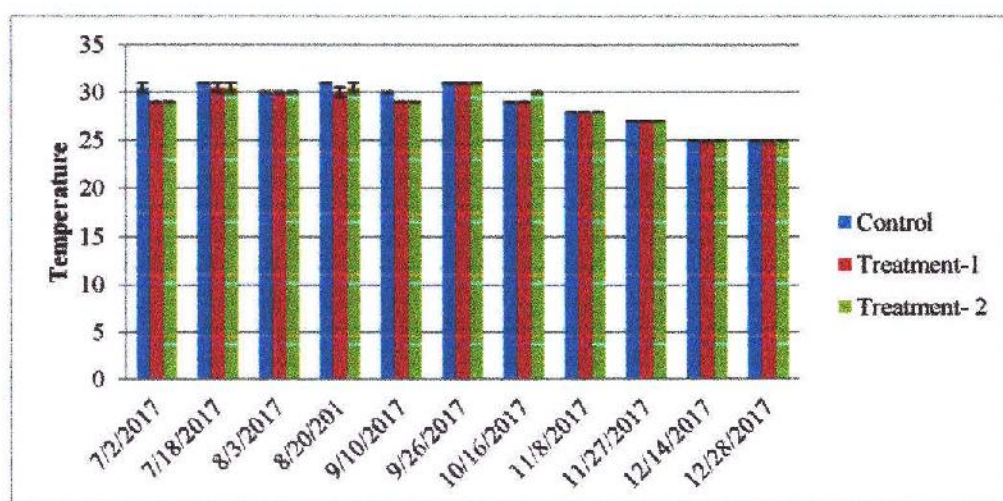


Fig. 04: Temperature in different treatments at different sampling period.

4.1.2 Dissolved oxygen (DO)

Dissolved oxygen (DO) concentration was ranged from 6 to 8 mgL⁻¹ in control, treatment-1 and treatment-2 throughout the study period. The highest mean DO concentration (mg L⁻¹) was recorded in the 3rd week of July and last week of September in the treatments but the lowest mean was recorded in December. There was no significance difference exists between control and two treatments. According to DoF (1996), the suitable ranges of DO for fish culture ranged

from 5.0 to 8.0 mg/L. Roy (2004) recorded 3.65 to 7.65 mg/L DO in carp-mola polyculture ponds in the rural farmers' pond.

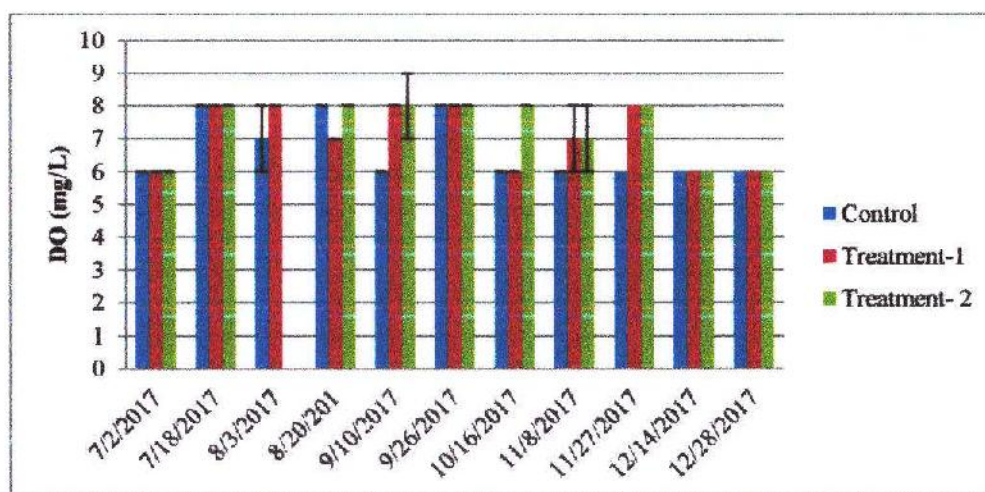


Fig. 05: DO in different treatments and different sampling period.

4.1.3 pH

pH was found to fluctuate from 7.5 to 8.7, 7.35 to 8.7 and 7.5-8.7 during the experiment in control, treatment-1 and treatment-2 respectively. The mean values of pH were 8.23 ± 0.44 , 8.32 ± 0.42 and 8.33 ± 0.37 in control, treatment-1 and treatment-2 respectively. The observed pH value was about similar with the findings of Hossain *et al* (1997).

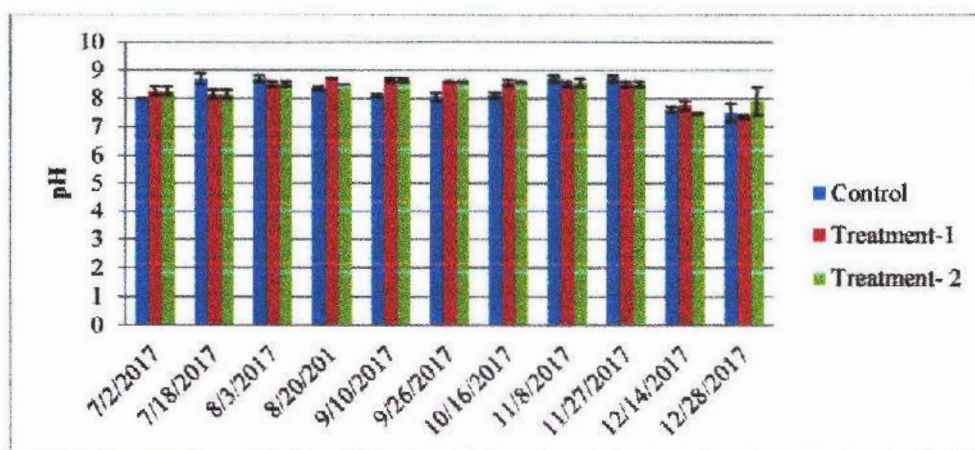


Fig. 06: pH in different treatments at different sampling period.

4.1.4 Alkalinity

Total alkalinity was found to vary from 200 to 275, 200 to 300 and 200-300mg L⁻¹ with the mean values of 220.45±24.54, 247.73±32.51 and 245.45±31.26 mg L⁻¹ during the experiment in control, treatment-1 and treatment-2 respectively. No significant (P>0.05) variation was observed for total alkalinity between control and treatments during the experimental period. The highest total alkalinity (300 mg/l) was observed in Treatment-1 and the lowest total alkalinity (200 mg/l⁻¹) was observed in both control and treatments. These values showed no significant differences between control and treatments.

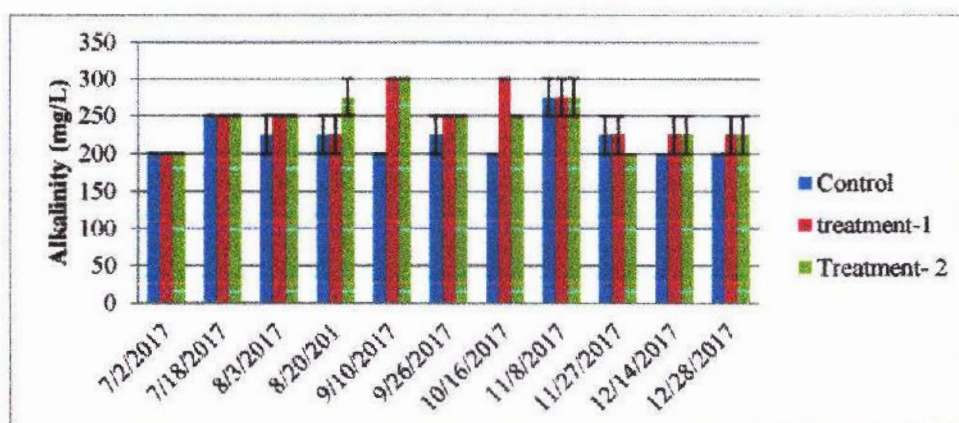


Fig. 07: Variation of alkalinity in different treatments at different sampling period.

4.1.5 Hardness

Hardness refers to the concentration of calcium and magnesium in water. The optimum range of hardness for polyculture farming is <120 ppm (Mazid, 2009). In this study, the observed hardness ranged from 155 to 170, 165 to 200 and 165-200 mg/l in control and treatment-1 and treatment-2 throughout the whole experiment period. The mean value of hardness was 164.09 ± 5.39 , 184.55 ± 11.93 and 183.18 ± 14.19 mg/l in control, treatment-1 and treatment-2 respectively which are more or less similar to the finding of Rahman (2005), Wahab *et al.*, (1995), Kohinoor (2001) and Asaduzzaman (2005) who recorded uniform range during their experiment. Significance difference of hardness was found between control and two treatments. Significantly high alkalinity was observed in the two treatments compaing to control probably for the inclusion of inorganic molicules.

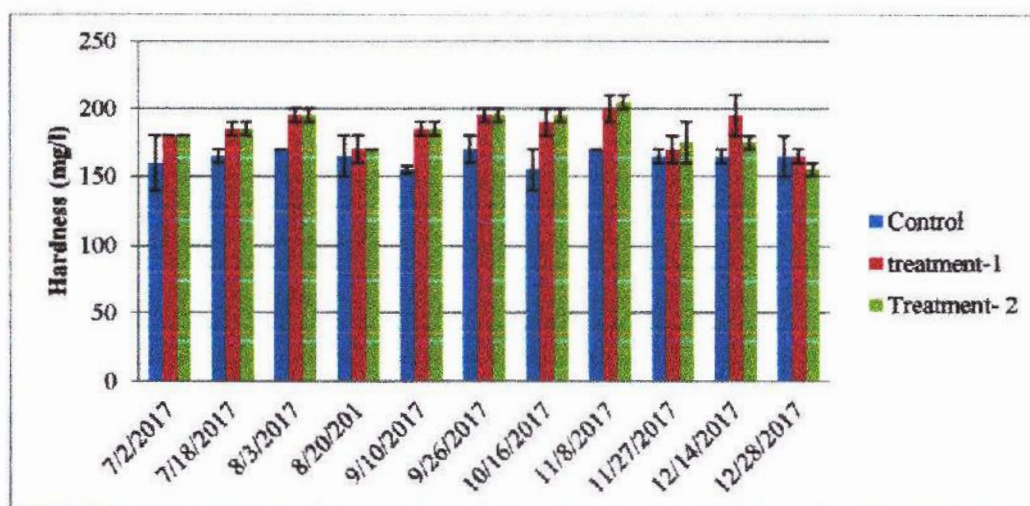


Fig. 08: Variation of Hardness in different treatments at different sampling period.

4.1.6 Ammonia

Ammonia in water exists in two forms, as ammonium ions (NH_4^+), which are nontoxic, and as the un-ionized toxic ammonia (NH_3). The desirable range of ammonia for polyculture farming is < 0.1 ppm and for prawn farming is 0 ppm. It was reported that the half of shrimp production was reduced in Bangladeshi farms due to the presence of ammonia at 0.45 ppm (Mazid, 2009). Total ammonia nitrogen ($\text{NH}_3\text{-N}$) concentration was found to vary from 0.038 to 0.138 mg L^{-1} during the experimental period in control and treatments respectively. The mean concentration of $\text{NH}_3\text{-N}$ was 0.07 ± 0.02 , 0.09 ± 0.03 and 0.09 ± 0.03 mg L^{-1} in control, treatment-1 and treatment-2 respectively. No significant difference ($P > 0.05$) was observed between control and treatments in one-way ANOVA test. The highest ammonia nitrogen concentration was observed during middle October in treatment while the lowest was control in July in control and treatment-2. Asaduzzaman *et al* (2006) recorded ammonia nitrogen value ranged from 0.203 to 0.569 mg/l .

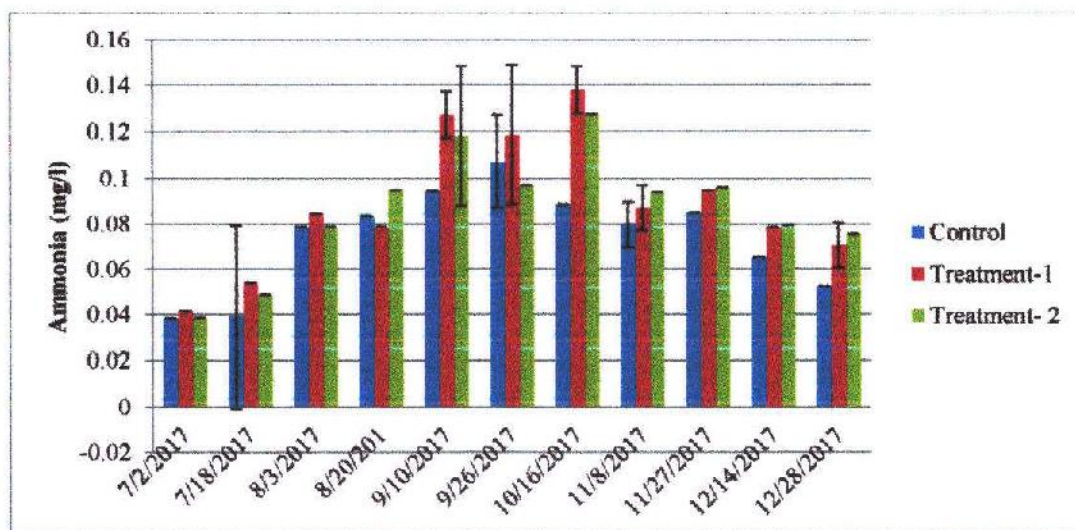


Fig. 09: Variation of ammonia in different treatments at different sampling period.

4.1.7 Nitrate

The concentration of nitrate-nitrogen ($\text{NO}_3\text{-N}$) was found to vary from 0.125 to 0.285 mg L^{-1} , with the mean values of 0.17 ± 0.03 , 0.21 ± 0.05 and 0.20 ± 0.05 mg L^{-1} in control, treatment-1 and treatment-2 respectively during the experimental period which are more or less similar to hoque *et al.* (2014) and kohinoor *et al.* (1998) who recorded 0.02 to 3.0 mg/L in pond in their experiments. The rang of $\text{NO}_3\text{-N}$ from 0.06 to 0.10 mg/L were found in pond waters by Azim Et *al.* (1995), Dewan *et al.* (1991) Hoque *et al.* (2014), Kohinoor (2000) and Wahab *et al.* (1995), which are more or less similar to the present study but was no significant difference ($P > 0.05$) was observed between control and two treatments. Though some fluctuations was observed in both control and two treatments throughout the study period.

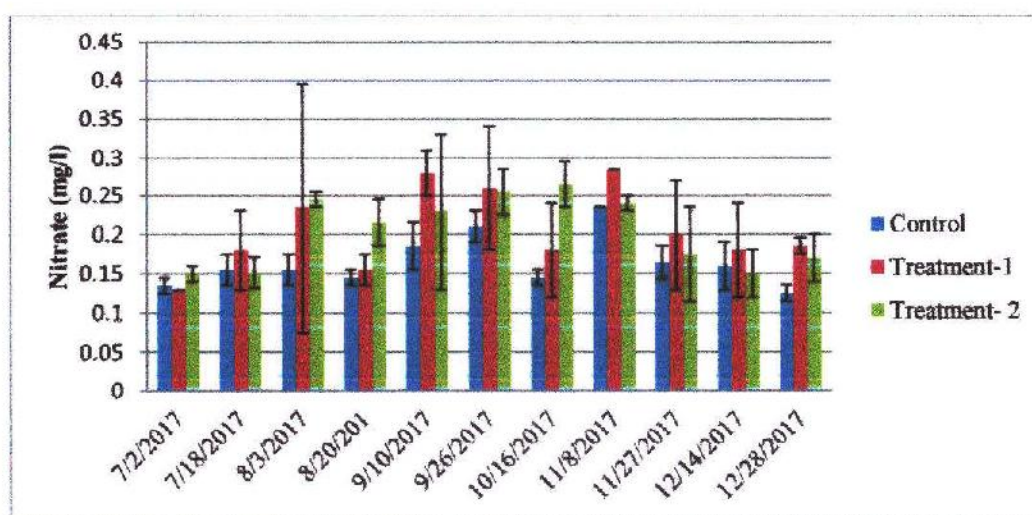


Fig. 10: Variation of nitrate in different treatments and different months.

4.1.8 Nitrite

The concentration of nitrite-nitrogen ($\text{NO}_2\text{-N}$) was found to be ranged from 0.0235 to 0.9715 mg l^{-1} in control and treatments respectively during the experimental period. one-way ANOVA showed no significant differences between control and two treatments.

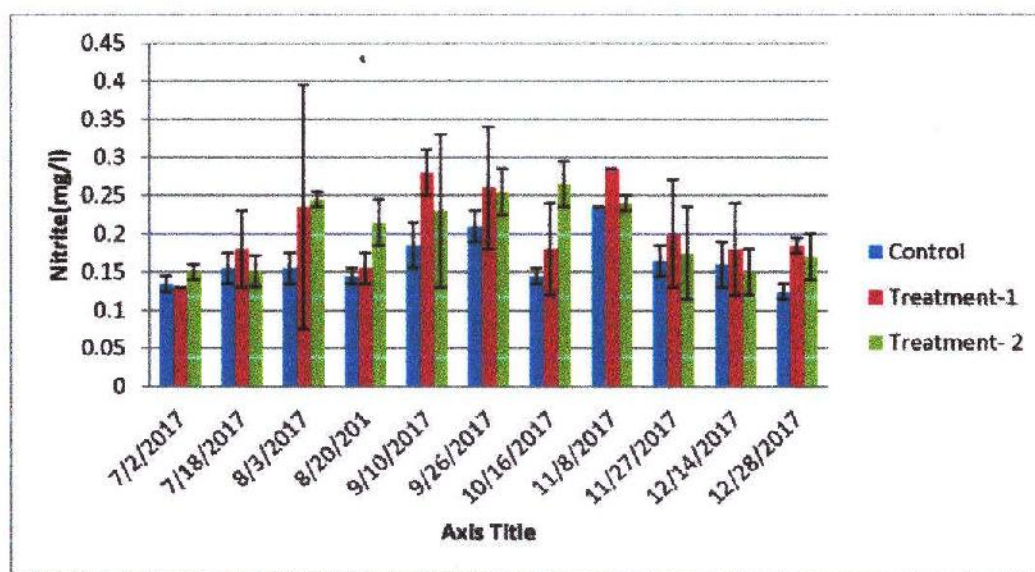


Fig. 11: Variation of nitrite in different treatments at different sampling period.

4.2 Growth and production of fish

Details of growth and production of prawn (*M. rosenbergii*), rohu (*L. rohita*) and mola (*A. mola*) were presented in figure 12, 13 and 14 and Table 03. Among all species, rohu (*L. rohita*) attained the maximum weight at harvest while the production of mola had been affected by prawn (*M. rosenbergii*).

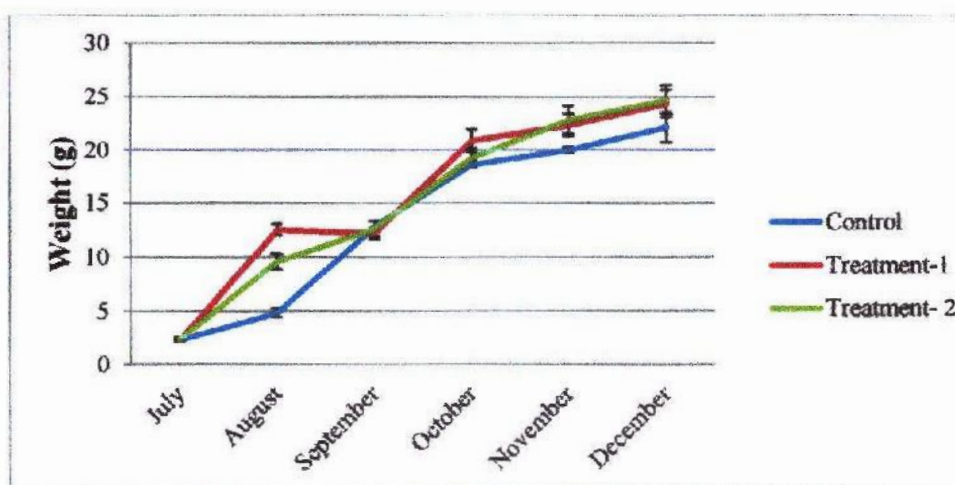


Fig. 12: Growth pattern of prawn (*M. rosenbergii*)

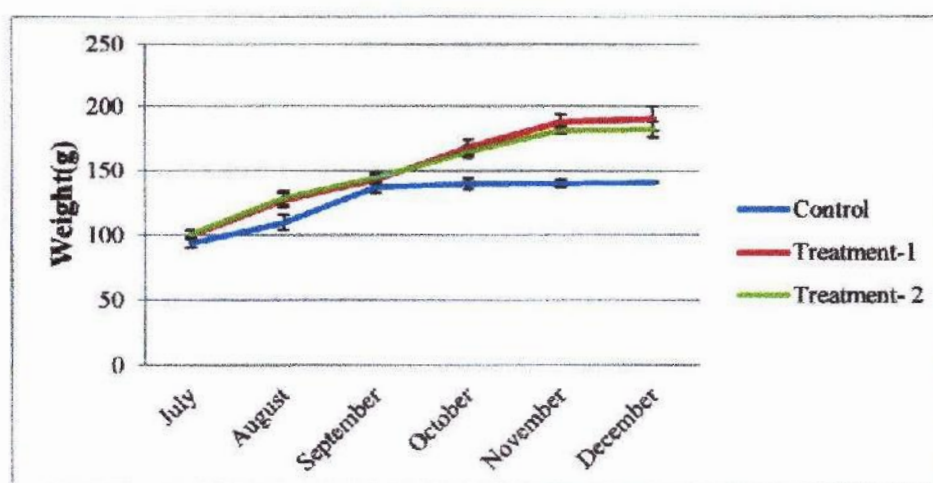


Fig. 13: Growth pattern of rohu (*L. rohita*)

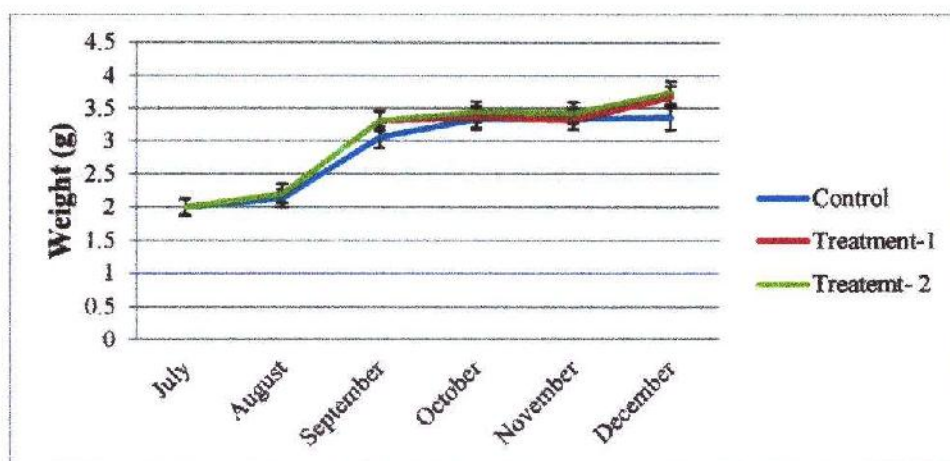


Fig. 14: Growth pattern of mola (*A. mola*)

4.2 Survival rate

The survival rates prawn (*M. rosenbergii*) and rohu (*L. rohita*) in control, treatment-1 and treatment-2 were 73.33, 89.58 and 86.25%, 78, 74 and 74% while mola (*A. mola*) were 10.67, 15.33 and 15.33% respectively. Perhaps mola (*A. mola*) fishes were consumed by prawn (*M. rosenbergii*). There was no significant difference in the survival rates of prawn (*M. rosenbergii*), rohu (*L. rohita*) and mola (*A. mola*) among control and two treatments.

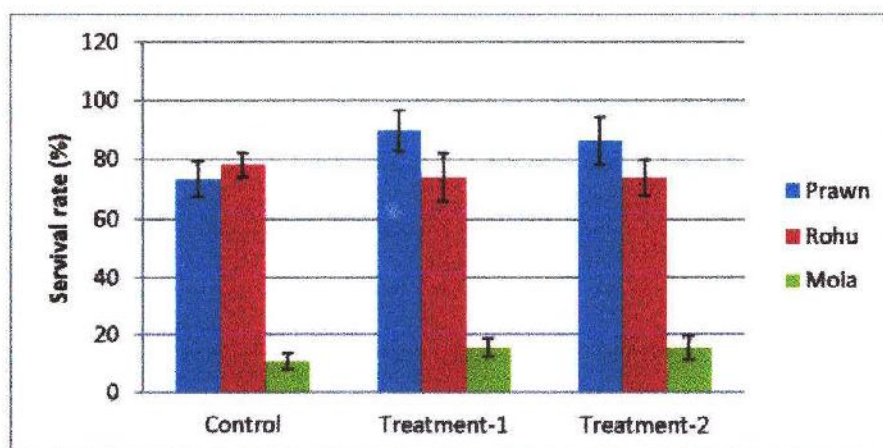


Fig. 15: Survival rate of fish species in different experimental conditions.

4.2.1 Final Production

The average final mean individual weights of prawn (*M. rosenbergii*), rohu (*L. rohita*) and mola (*A. mola*) in treatment-1 were 24.35, 190.6, 3.67g and treatment-2 were 24.7, 182.2, 3.73g while 22.1, 141.1, 3.36 g were respectively in control. (Fig. 16) The final harvest weights of mola (*A. mola*) in treatment-1 and treatment-2 was more or less similar as control. The survival rate of prawn (*M. rosenbergii*) in treatment-2 was lower than treatment-1. So the final production of treatment-1 was more than treatment-2.

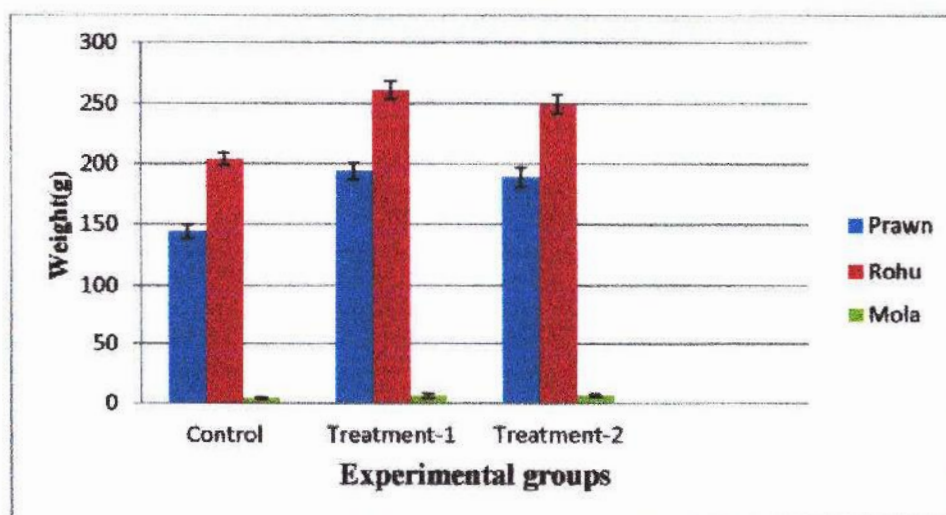


Fig. 16: Final production of prawn, rohu and mola at different experimental conditions.

Production after six months of culture was 351.8, 461.5 and 445.3 kg/ha in control, treatment-1 and treatment-2 respectively (Table 3). Significant difference was found in the production of Prawn (*M. rosenbergii*), Rohu (*L. rohita*) and Mola (*A. mola*) among control and two treatments. Thilsted *et al.* (2003) stated that, total fish production was 2560, 2412 and 2176 kg ha⁻¹ in three treatments respectively. Wahab *et al* (2008) recorded an integrated aquaculture of freshwater

prawn (*M. rosenbergii*), and mola (*A. mola*) in different plot with better average production, 222 to 388 kg ha⁻¹, 51 to 68 kg ha⁻¹.

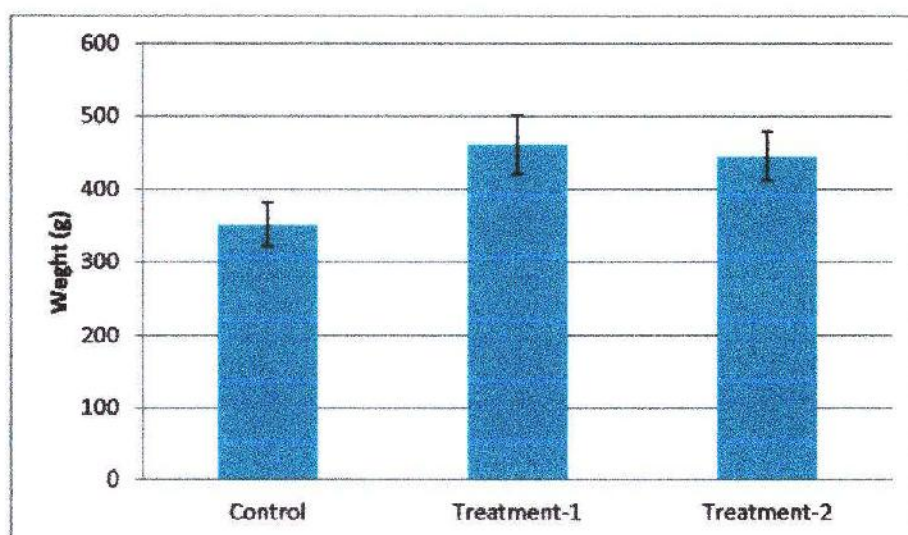


Fig. 17: Total production in two treatments and control ponds.

Table 03: Survival and production of prawn, rohu and mola under two treatments

Treatment	At stocking			At harvest			Survival		Production	
	Species	Av.	No. of fish	Total wt	Av. Final wt	No of fish	Rate (%)	Rate (%)	Species	Total
		Initial	stocked	(g)	(g)	recovered		(kg/ha)	wise	
		wt (g)								
Control	Prawn	2.32	240	556.8	22.1	176	73.33			144
	Rohu	94.13	50	4706.5	141.1	39	78			203.8
	Mola	2	300	600	3.36	32	10.67			4
Treatment-1	Prawn	2.32	240	556.8	24.35	215	89.58			194
	Rohu	100.3	50	5015	190.6	37	74			261.2
	Mola	2	300	600	3.67	46	15.33			6.3
Treatment-2	Prawn	2.44	240	585.6	24.7	207	86.25			189.3
	Rohu	100.85	50	5042.5	182.2	37	74			249.6
	Mola	2	300	600	3.73	46	15.33			6.4
										445.3



Chapter.....5

Conclusion

5. Conclusion

In conclusion, fertilizers indirectly played a major role by increasing production of fishes in T-1 and T-2 than control. Use of macro-nutrients enriched Treatment-1 gave better overall production of fishes comparing to the Treatment-2 with micro-nutrients based commercial fertilizers. However including mola could to maximize the production of prawn and rohu in fertilizer included polyculture system though mola production may be affected by the other fishes. Therefore, inclusion of traditional inorganic fertilizers with the recommended doses of this study might be helpful for carp-prawn polyculture to meet economical and nutritional benefits.

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Chapter.....6

References

6. References

- Ahmed, N., Demaine, H. and Muir, J. (2008). Freshwater prawn farming in Bangladesh: history, present status and future prospects. *Aquaculture Research*, 39(8), pp.806-819.
- Akand, A. M. (1986). Effects of inorganic fertilizers on water quality and fish production in carp ponds. *Bangladesh Journal of Fisheries*.
- Akand, A. M. and Hasan, M. R. (1992). Status of freshwater prawn (*Macrobrachium spp.*) culture in Bangladesh. *Freshwater Prawns (ed. by EG Silas)*, 33-41.
- Allen, P. (1993). Effects of acute exposure to cadmium(II) chloride and lead(II) chloride on the haematological profile of *Oreochromis aureus* (Steindachner). *Comparative Biochemistry and Physiology, C*, 105(2), 213-217.
- Asaduzzaman, M., Yang, Y., Wahab, M. A., Diana, J.S. and Ahmed, Z.F. (2006). Farming system of giant freshwater prawn (*Macrobrachium rosenbergii*) in Bangladesh: a combination of tradition and technology. Proceeding of the WAS conference held on 9-13 may, 2006 in Florence, Italy.
- Azim, M. E., Talukder, G. S., Wahab, M. A., Haque, M. M, and Haq, M. S. (1995). Effect of liming and maintenance of total hardness levels on fish production in fertilized ponds. *Journal of agricultural Progress*, 6(2), 7-14.
- Bangladesh, I. U. C. N. (2000). In Red Book of Threatened Fishes of Bangladesh, edited by Mahmud-ul-Ameen, Md. Anwarul Islam, and Ainun Nishat. *The World Conservation Union*, xi.

- Boyd, C. E. (1976). Nitrogen fertilizer effects on production of Tilapia in ponds fertilized with phosphorus and potassium. *Aquaculture*, 7(4), 385-390.
- Boyd, C. E. (1982). Water quality management for pond fish culture. *Elsevier Scientific Publishing Company*.
- Brucka-Jastrzębska, E., Kawczuga, D., Protasowicki, M. and Rajkowska, M. (2010). Effect of culture conditions on magnesium and zinc concentrations in muscles of freshwater fish. *Journal of Elementology*, 15(2): 239-250.
- Brucka-Jastrzębska, E., Kawczuga, D., Rajkowska, M, and Protasowicki, M. (2009). Levels of microelements [Cu, Zn, Fe] and macroelements [Mg, Ca] in freshwater fish. *Journal of Elementology*, 14(3), 437-447.
- Bury, N. R., Walker, P. A, and Glover, C. N. (2003). Nutritive metal uptake in teleost fish. *Journal of experimental biology*, 206(1), 11-23.
- Chakrabarty, R.D., D.S. Murty, P.K., Sen, A.C., Nandy, and Chakrabarty, D.P. (1976). Short-term rearing of Indian and exotic carps. *Journal of Inland Fisheries Society, India* 8: 179-183.
- Comhaire, S., Blust, R., Van Ginneken, L., D'haeseleer, F, and Vanderborght, O. (1997). Calcium influences radio-cobalt uptake by the common carp, *Cyprinus carpio*. *Studies in Environmental Science*, 68, 321-328.

- Cooper C.A., Bury N.R. (2007). The gills as an important uptake route for the essential nutrient iron in freshwater rainbow trout *Oncorhynchus mykiss*. *Journal of fish Biology*, 71: 115-128.
- Cowey C. B., Knox D., Adron J.W., George S, and Pirie, B. (1977). The production of renal calcinosis by magnesium deficiency in rainbow trout (*Salmo gairdneri*). *British journal of nutrition*, 38: 127-135.
- Davis D.A., Gatlin D.M. (1996). Dietary mineral requirements of fish and marine crustacean. *Reviews of fisheries science*, 4(1): 75-99.
- Debeljak, L., Turk, M., Fasaic, K, and Popovic, J. (1990). Mineral fertilizers and fish production in carp ponds: In *Symposium on Production Enhancement in Still-Water Pond Culture, Prague (Czech Republic), 15-18 May 1990. Research Institute of Fish Culture and Hydrobiology*.
- Dewan, D., Wahab, M. A., Beveridge, M. C. M., Rahman, M. H, and Sarkar, B. K. (1991). Food selection, electivity and dietary overlap among planktivorous Chinese and Indian major carp fry and fingerlings grown in extensively managed, rain-fed ponds in Bangladesh. *Aquaculture Research*, 22(3), 277-294.
- DoF, (2009). *Matsha pakkha shankaln- 2009*. Department of Fisheries, Bangladesh. pp. 13-120.
- DoF, (2017). Fishery statistical yearbook of Bangladesh 2016-2017. Dhaka, Bangladesh: Department of Fisheries, Ministry of Fisheries and Livestock, Government of Peoples Republic of Bangladesh.

- Ekram, (2002). The Potential of Periphyton-based Aquaculture Systems. An unpublished Ph.D Dissertation, Wageningen University, Netherlands, 72 p.
- FAO, (2013). World Aquaculture and Fisheries Statistics. FAO Publication, Rome, Italy.
- FAO, (2016). The State of World Fisheries and Aquaculture. FAO Publication, Rome, Italy.
- FAO, (2017). FAO Aquaculture News Letter (FAN). FAO Publication, Rome, Italy.
- Felts, R. A., Rajts, F, and Akhteruzzaman, M. (1996). Small indigenous fish species culture in Bangladesh (Technical Brief), IF ADEP sub-project-2. *Development of inland fisheries*, 41.
- Garg, S. K, and Bhatnagar, A. (2000). Effect of fertilization frequency on pond productivity and fish biomass in still water ponds stocked with *Cirrhinus mrigala* (Ham.). *Aquaculture Research*, 31(5), 409-414.
- Gerking, S. D. (1966). The biological basis of freshwater fish production. a symposium sponsored by sectional committee on productivity of freshwater communities of the international biological programme the university reading england 1-6 september 1966.
- Green, B. W., Phelps, R. P, and Alvarenga, H. R. (1989). The effect of manures and chemical fertilizers on the production of *Oreochromis niloticus* in earthen ponds. *Journal of Aquaculture*, 76(1-2), 37-42.
- Green, E.P., Harris, R.P. and Duncan, A. 1992. The production and ingestion of faecal pellets by nauplii of marine calanoid copepods. *Journal of Plankton Research* 14 (12): 1631-1643.

- Handy, R. D, and Penrice, W. S. (1993). The influence of high oral doses of mercuric chloride on organ toxicant concentrations and histopathology in rainbow trout, *Oncorhynchus mykiss*. *Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology*, 106(3), 717-724.
- Hellawell, J. M. (1988). Toxic substances in rivers and streams. *Environmental Pollution*, 50(1-2), 61-85.
- Hoq, M. E., Islam, M. M, and Hossain, M. M. (1996). Polyculture of freshwater prawn (*Macrobrachium rosenbergii*) with Chinese and Indian carps in farmer's pond. *Journal of Aquaculture in the Tropics*, 11, 135-142.
- Hoque, M. M., Motin, M. A., Maya, M., Uddin, M. R, and Chowdhury, A. J. K. (2014). Water Quality and Plankton Composition of Amblypharyngodon mola Monoculture Fish Pond in Bangladesh. *International Journal of Emerging Trends in Science and Technology*, 1(07).
- Hossain, M. A., Rahmatullah, S. M., Islam, M. S., Kabir, A. K. M. A, and Dewan, S. (1997). Impact of chapila (*Gudusia chapra* Ham.) on growth of carps in polyculture. *Bangladesh Journal of Fisheries Research*, 1(2), 19-23.
- Kalantarian, S. H., Rafiee, G. H., Farhangi, M, and Amiri, B. M. (2013). Effect of different levels of dietary calcium and potassium on growth indices, biochemical composition and some whole body minerals in rainbow trout (*Oncorhynchus mykiss*) fingerlings. *Journal of Aquaculture Research and Development*, 4(3).

- Kamunde, C., Grosell, M., Higgs, D, and Wood, C. M. (2002). Copper metabolism in actively growing rainbow trout (*Oncorhynchus mykiss*): interactions between dietary and waterborne copper uptake. *Journal of Experimental Biology*, 205(2), 279-290.
- Kohinoor, A. H. M. (2000). Development of culture technology of three small indigenous fish mola (*Amblypharyngodon mola*), punti (*Puntius sophore*) and chela (*Chela cachius*) with notes on some aspects of their biology. A PhD dissertation, Department of Fisheries Management, Bangladesh Agricultural University, Mynensingh, 365.
- Kohinoor, A. H. M., Islam, M. L., Wahab, M. A, and Thilsted, S. H. (1998). Effect of mola (*Amblypharyngodon mola* Ham.) on the growth and production of carps in polyculture. *Bangladesh Journal of Fisheries Research*, 2(2), 119-126.
- Kohinoor, A. H. M., Wahab, M. A., Islam, M. ., and Thilsted, S. H. (2001). Culture potentials of mola (*Amblypharyngodon mola*), chela (*Chela cachius*) and punti (*Puntius sophore*) under monoculture system. *Bangladesh Journal of Fisheries Research*, 5(2), 123-134.
- Kopp, R., Lang, S., Brabec, T, and Mares, J. (2013). Influence of physicochemical parameters of water on plasma indices in brook trout (*Salvelinus fontinalis* Mitchill) reared under conditions of intensive aquaculture. *Journal of acta veterinaria*, 82(40), 367-373.
- Kroupova, H., Machova, J, & Svobodova, Z. (2005). Nitrite influence on fish: a review. *Journal of veterinarni medicina-praha-*, 50(11), 461.
- Kunda, M., Azim, M. E., Wahab, M. A., Dewan, S., Roos, N, and Thilsted, S. H. (2008). Potential of mixed culture of freshwater prawn (*Macrobrachium rosenbergii*) and self-

recruiting small species mola (*Amblypharyngodon mola*) in rotational rice–fish/prawn culture systems in Bangladesh. *Aquaculture Research*, 39(5), 506-517.

Mahean Haque, S., Wahab, M. A., Wahid, M. I, and Haq, M. S. (1998). Impacts of Thai silver barb (*Puntius gonionotus* Bleeker) inclusion in the polyculture of carps. *Bangladesh Journal of Fisheries Research*, 2(1), 15-22.

Mukherjee, S, and Kaviraj, A. (2009). Evaluation of growth and bioaccumulation of cobalt in different tissues of common carp, *Cyprinus carpio* (Actinopterygii: Cypriniformes: Cyprinidae), fed cobalt-supplemented diets. *An international journal for ichthyology and fisheries*, 39(2).

Mazid, M.A. 2009. Training manual on water quality management in shrimp farm Bangladesh Quality Support Programm- Fisheries, UNIDO, DHAKA, Bangladesh. 108 pp.

Niyogi, S., Pyle, G. G, and Wood, C. M. (2007). Branchial versus intestinal zinc uptake in wild yellow perch (*Perca flavescens*) from reference and metal-contaminated aquatic ecosystems. *Canadian Journal of Fisheries and Aquatic Sciences*, 64(11), 1605-1613.

Rahman, M. M., Verdegem, M. C. J., Nagelkerke, L. A. J., Wahab, M. A., Milstein, A, and Verreth, J. A. J. (2006). Growth, production and food preference of rohu *Labeo rohita* (H.) in monoculture and in polyculture with common carp *Cyprinus carpio* (L.) under fed and non-fed ponds. *Aquaculture*, 257(1-4), 359-372.

Roy, N. C. (2004). Effects of Carp-SIS Polyculture System on Pond Fish Production, Nutritional Security and Socioeconomic Benefit of the Rural People. (Doctoral dissertation, Ph. D.

Dissertation, Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh).

Schroeder, G. L. (1980). Fish farming in manure-loaded ponds [in Israel]. In *ICLARM Conference Proceedings (Philippines)*.

Shi, J, and Camus, A. C. (2006). Hecpidins in amphibians and fishes: antimicrobial peptides or iron-regulatory hormones?. *Developmental and Comparative Immunology*, 30(9), 746-755.

A. G. (1987). The nutrition and feeding of farmed fish and shrimp: a training manual. 1: The essential nutrients.

Thilsted, S. H., Khatoon, H., Wahab, M. A, and Roy, N.C. (2003). Economics of carp SIS polyculture in Rural Farmers Pond. *Pakistan Journal of Biological Sciences*. 6 (1): 61-64.

Tidwell, J. H, and Allan, G. L. (2001). Fish as food: aquaculture's contribution: Ecological and economic impacts and contributions of fish farming and capture fisheries. *EMBO reports*, 2(11), 958-963.

Tripathi, S. D. (1983). Recent advances in composite fish culture. *Lectures on Composite fish Culture and its Extension in India*.

Wahab, M. A. (1994). Compatibility of silver carp in the polyculture of cyprinid fishes. *Journal of agricultural Progress*, 5, 221-227.

Wahab, M. A., Ahmed, Z. F., Islam, M. A., Haq, M. S, and Rahmatullah, S. M. (1995). Effects of introduction of common carp, *Cyprinus carpio* (L.), on the pond ecology and growth of fish in polyculture. *Aquaculture Research*, 26(9), 619-628.

Wahab, M.A., Kunda, M., Azim, D.E., Dewan, S, and Thilsted, S. H. (2008). Evaluation of freshwater prawn-small fish culture concurrently with rice in Bangladesh. *Aquaculture research*, 39 1524-1532.

Watanabe T., Satoh S, and Takeuchi T. (1988). *Availability of minerals in fish meal to fish*. Department of Aquatic Biosciences, Tokyo University of Fisheries, *Asian Fisheries Science*, 175-195.

Watanabe, T., Kiron, V, and Satoh, S. (1997). Trace minerals in fish nutrition. *Aquaculture*, 151(1-4), 185-207.