EFFECT OF STOCKING DENSITY ON THE GROWTH PERFORMANCES OF SHING (*Heteropneustes fossilis*) UNDER POLYCULTURE SYSTEM IN SEASONAL MINI PONDS OF NORTHERN REGION OF BANGLADESH

A Thesis

By

MD. SOHEL RANA

Examination Roll No. 1605208

Session: 2016-2017

Semester: January-June, 2017

MASTER OF SCIENCE (MS)

IN

AQUACULTURE

DEPARTMENT OF AQUACULTURE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
DINAJPUR

JUNE 2017

EFFECT OF STOCKING DENSITY ON THE GROWTH PERFORMANCES OF SHING (*Heteropneustes fossilis*) UNDER POLYCULTURE SYSTEM IN SEASONAL MINI PONDS OF NORTHERN REGION OF BANGLADESH

A Thesis

By

MD. SOHEL RANA

Examination Roll No. 1605208

Session: 2016-2017

Semester: January-June, 2017

Submitted to the Department of Aquaculture

Hajee Mohammad Danesh Science and Technology University, Dinajpur

In partial Fulfillment of the Requirements

For the degree of

MASTER OF SCIENCE (MS)

IN

AQUACULTURE

DEPARTMENT OF AQUACULTURE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
DINAJPUR

JUNE 2017

EFFECT OF STOCKING DENSITY ON THE GROWTH PERFORMANCES OF SHING (*Heteropneustes fossilis*) UNDER POLYCULTURE SYSTEM IN SEASONAL MINI PONDS OF NORTHERN REGION OF BANGLADESH

A Thesis

By

MD. SOHEL RANA

Examination Roll No. 1605208

Session: 2016-2017

Semester: January-June, 2017

Approved as to style and contents by

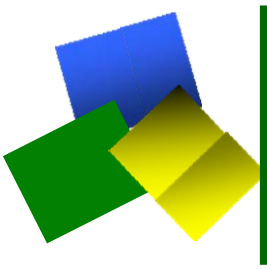
(Prof. Dr. Mst. Nahid Akter)	**(Dr. Khondaker Rashidul Hasan)**
Supervisor	**Co-supervisor**

(Prof. Dr. Mst. Nahid Akter)
Chairman
Examination Committee
&
Chairman

Department of Aquaculture

Hajee Mohammad Danesh Science and Technology University, Dinajpur

June 2017



DEDICATED TO MY
BELOVED PARENTS

DECLARATION

I declare that this MS thesis entitled Effect of Stocking Density on the Growth Performances of Shing (*Heteropneustes fossilis*) under Polyculture System in Seasonal Mini Ponds of Northern Region of Bangladesh, which I submit to Department of Aquaculture, was carried out by me for the degree of Masters in Aquaculture under the guidance and supervision of (Dr. Mst. Nahid Akter), (Professor) Department of Aquaculture, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

Furthermore, I took reasonable care to ensure that the work is original, and has not been taken from other sources except where such work has been cited and acknowledged within the text.

The Author

ACKNOWLEDGEMENTS

All the gratefulness of the author goes to the Almighty Allah, who has kindly made the author successful to pursue the higher study and to complete the research work and thesis for the degree of Master of Science (MS) in Aquaculture.

The author expresses his sincere and heartfelt gratitude, the deepest sense of respect, immense indebtedness and the best regards to his research supervisor, Dr. Mst. Nahid Akter, professor, Department of Aquaculture, Hajee Mohammad Danesh Science and Technology University, Dinajpur for her affectionate encouragement, scholastic supervision, constructive criticism, intellectual guidance, valuable suggestions and affectionate feeling in planning, conducting and completing the study.

The author also expresses his heartiest appreciation and deep sense of respect to his research co-supervisor Dr. Khondaker Rashidul Hasan, Senior Scientific Officer, Bangladesh Fisheries Research Inistitute, Freshwater Sub-station, Saidpur, Nilphamari, for his co-operation, suggestions and valuable comments to overcome many errors in upgrading the quality of the research.

The author feels pleasure to extend his heartiest respect and cordial thanks to all respected teachers of the Department of Aquaculture, Hajee Mohammad Danesh Science and Technology University, Dinajpur, for their valuable teaching, inspiration and encouragement during the whole course of study.

The author indebted to the assistance rendered by the staff of the Aquaculture Department, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh Fisheries Research Inistitute, Freshwater Sub-station, especially Scientific Officer Saokat Ahamed, Saidpur, Nilphamari and also thanks the fish farmers who have helped during the study period.

The author humbly desires to say thanks to all his friends and well-wishers, especially Najida Khatun for their help in active encouragement and inspiration.

The author acknowledges with great regards and pleasure, his deepest sense of gratitude and thanks to his beloved parents, brothers, sister and other relatives, who sacrificed a lot during his studies and were the constant source of inspiration.

Finally, the authors express to his cordial thanks to IAPP (Integrated Agricultural Productivity Project) under BFRI (Bangladesh Fisheries Research Institute) Sub-station, Saidpur, Nilphamari for providing all types of assistance of this research.

The Author

TABLE OF CONTENTS

TABLE OF CONTENTS (Contd.)

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

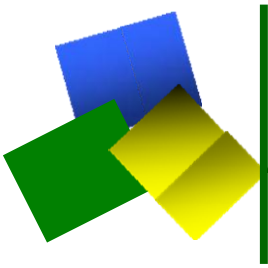
ABBREVIATIONS

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemist
BAU	Bangladesh Agricultural University
BBS	Bangladesh Bureau of Statistics
BCR	Benefit Cost Ratio
BFRI	Bangladesh Fisheries Research Institute
CM	Centimeter
DEC	Decimal
DMRT	Duncan's Multiple Range Test
DO	Dissolved Oxygen
DoF	Department of Fisheries
EDTA	Ethylene-Diamine-Tetra-Acetic acid
FAO	Food and Agriculture Organization
FRSS	Fisheries Research Survey System
g	gram
GDP	Gross Domestic Product
GIFT	Genetically Improved Farmed Tilapia
Ha	Hectare
HSI	Hepatosomatic Index
IPF	Intraperitoneal Fat
IUCN	International Union of Conservation of Nature
INFS	Institute of Nutrition and Food Science
Kg	Kilogram
L	Liter
mg	milligram
MT	Metric Tons
PPM	Parts Per Million
R_1	Replication-1
R_2	Replication-2
R_3	Replication-3
SE	Standard Error
SGR	Specific Growth Rate
SPSS	Statistical Package for Social Science
T_1	Treatment-1
T_2	Treatment-2
T_3	Treatment-3
VSI	Viscerosomatic index
USA	United States of America

ABSTRACT

The present study was carried out to evaluate the effect of stocking density on the growth performance and water quality parameters of shing (*Heteropneustes fossilis*) polyculture system at Saidpur and Dimla Upazila of Nilphamari district and Taragonj Upazila of Rangpur district, for a period of 150 days from 30 March 2016 to 30 August 2016. *H. fossilis* with an average weight of around 3 g were stocked in nine uniform earthen ponds (around 10 decimal) at three different stocking densities of 500 (T_1), 600 (T_2) and 700 dec^{-1} (T_3) together with *Clarias batratus*, *Oreochromis niloticus* and *Barbodes gonionotus* at a ratio of 5:2:1. All the experimental fish were fed with a commercial feed (Lily fish feed) containing 35.53% protein twice daily at 5-10% body weight of *H. fossilis.* During the study period, the water quality parameters were evaluated and the results revealed that significantly lowest ($P<0.05$) total alkalinity, hardness and ammonia content were noted in the lowest stocking density (T_1) compared to those that were stocked at highest stocking density (T_3). At the end of the feeding trial, weight gain (62.15±0.64g), percent weight gain (2135.46±5.05 %), average daily gain (0.41±0.01 % day^{-1}) and SGR (2.04±0.01 % day^{-1}) were significantly improved ($P<0.05$) in the treatment of T_1 compared with the other treatment groups. Significantly highest ($P<0.05$) survival (%) of shing was also found in the treatment of T_1 (77.93±0.94%) compared to the remaining treatments. Considering the survival highest gross production (kg dec^{-1}) was observed in T_2 (26.50±0.65) followed by T_1 (25.38±0.55) and T_3 (24.43±0.26). The benefit-cost ratio (BCR) result revealed that the best BCR was obtained in the treatment of T_1. Therefore, the results of this study indicated that the lowest stocking density (500 fingerlings dec^{-1}) seems to have more positive effect on the growth performances of shing (*H. fossilis*) polyculture in the seasonal farmer's mini ponds.

KEYWORDS: Stocking density, growth performance, *Heteropneustes fossilis*, polyculture, seasonal ponds



CHAPTER 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

Bangladesh is ranked fourth position in inland fishery production just after China, India and Myanmar and fifth position in closed waters (FRSS, 2016). In the life and lifestyle of the people of Bangladesh, Fisheries sector are inseparable. In 2014-2015, fisheries sector contributes 3.69% to the national gross domestic product (GDP) and almost one-fourth (22.60%) to the agricultural GDP of Bangladesh (FRSS, 2016). About 17.80 million people (full time and part time) are employed by this sector (DoF, 2015).

The aquatic biodiversity of the country rich and attributed to the world's one of the largest wetlands (Bengal Delta) and three large river systems (Brahmaputra, Ganges and Jamuna) that flow from the Himalayan Mountains into the Bay of Bangal. We have huge inland fisheries resources, which supply fish and other aquatic animals and plants to millions of people living in the Delta (Hossain, 2014).

Rangpur region is a climate prone area. The capacity of water retention of the pond is decreasing day by day. For this reason the number of seasonal pond (60%, BBS, 2000) is increasing. In northern region of Bangladesh, there were remain more than 60% seasonal small ditches or ponds which retain water only 4-6 months round the year (Rahman *et al.*, 2013), while 40% retained for 6-9 months in a year and even more in some areas. Normally, people use these small water bodies for their household activities but some are still abandoned due to their derelict and marshy nature.

The farmers in this region believe that these waters could not be utilized for fish production purpose due to their seasonal nature, but actually they hold tremendous potential for adopting intensive culture of species having shorter life cycle, hardy and

survive in adverse ecological condition, high nutritive value as well as high market price. Nilphamari district was such an area for all kinds and types of fish farmers for increasing fish production and income generation where this culture technique would be the most wanted and effective.

Polyculture or composite fish culture is the system in which fast growing compatible species with different feeding habits are grown in the same pond (Jhingran, 1975). Polyculture management technique is based on the relationship between fishes at different levels of the food chain and environment. The outcome of fish production from polyculture systems depends on the species combinations and their stocking densities. Polyculture is one of the major culture techniques that have been used traditionally in Bangladesh, where carp polyculture were practiced in the farmer's ponds for rapid growth and maximum production. However, there was no information on polyculture practice of *H. fossilis* (Heteropneustidae) in Bangladesh.

The stinging catfish, *H. fossilis* (Bloch) was commercially as well as aquaculturally an important species in many Asian countries (Akand *et al.*, 1991) and it was an indigenous species to Indo-Pak-Bangladesh subcontinent. The local name of *H. fossilis* is shing (IUCN, 2000). *H. fossilis* is an indigenous stinging catfish of South-East-Asian region. The range encompasses India, Thailand, Bangladesh, Pakistan, Nepal, Srilanka, Myanmar, Indonesia and Cambodia (Burgess, 1989).

H. fossilis is locally known as shingi or shing in different parts of Bangladesh. It is hardy and amenable to a high stocking rate and utilizes atmospheric oxygen for respiration and thus makes them ideal species for aquaculture (Vijayakumar *et al.*, 1998). It is not only recognised for its excellent taste and market value but is also highly sought after for its nutritional and medicinal benefits. The species has high content of iron (226 mg $(100\ g)^{-1}$)

and fairly high content of calcium compared to many other freshwater fishes (Saha and Guha, 1939). Due to its high nutritive value the fish was recommended in the diet of the sick and the convalescents (Kohli and Goswami, 1989). Being a lean fish it is very suitable for people for whom animal fats are undesirable (Rahman *et al.*, 1982).

Shing are commonly found in open waters (streams, lakes, floodplains and beels), paddy fields and swamps of Bangladesh and its preferred habitats are heavily-vegetated, stagnant waters. This fish is popular particularly because it can be cultivated in swampy areas and derelict water bodies without involving costly reclamation. Unlike water-breathing fish, air-breathing fish can be easily stored and transported live to consumers. Thus, this species is ideal for wastewater aquaculture (Tharakan and Joy, 1996). They can survive for a few hours out of the water due to the presence of accessory respiratory organs.

Indiscriminate destructive practices have caused havoc to aquatic bio-diversity (Hussain and Mazid, 2001) in Bangladesh. International Union of Conservation of Nature (IUCN, 2000) enlisted *H. fossilis* in the "not threatened" category in Bangladesh. But in recent years, the fish has become gradually been endangered as the natural habitats and breeding grounds of this fish has been severely degraded due to over exploitation, ecological changes, reduction of water bodies, application of pesticides in rice cultivation, release of chemical effluents from industrial plants and hydrological changes due to construction of flood control infrastructure (Khan *et al.*, 2003 and Kohinoor *et al.*, 2012).

The native species are threatened now due to poorly planned water management policy for irrigation, over exploitation, illegal fishing and various ecological changes in its natural habitat (Chakraborty, 2010). Considering the importance of these species from

the nutritional, economic and biodiversity point of view, appropriate culture technologies for *H. fossilis* are needed to meet the dietary demand and ultimately more of these tasty fishes will be available for the rural people of Bangladesh. For large scale production of these fishes, comprehensive information on culture technologies is required.

This study was expected to provide financial support for poor fish farmers and a source of quick return of money also. From the aquaculture perspective, those water bodies have a great potentiality but expect for a few which were brought under for culturing fish species, which have short life cycle and faster growth and require low inputs, such as shing (*H. fossilis*), genetically improved farmed tilapia (GIFT), silver barb (*B. gonionotus*) and magur (*C. batrachus*).

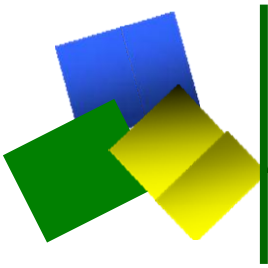
To ensure the proper utilization of these ponds, the culture of short-cycle species should be introduced which would be enhanced to get maximum production. The popular belief was that there were special nutritive and medicinal quality in those fishes which were good for patients and convalescents by Mookerjee and Mazumder (1950). But non-availability of quality fish seed and lack of knowledge of appropriate culture techniques are the major constrains to disseminate the culture technologies in Bangladesh especially in Northern region.

The present experiment was conducted to study the effects of stocking density on the growth performances of shing in ponds, to compare the production of shing at different stocking densities under polyculture system and to assess the water quality parameters. Hence, the present study has been designed and proposed to demonstrate these research results at farmer's ponds as well as to validate the technologies.

Objectives

- To assess the effect of stocking density on the growth performances and proximate composition of shing (*H. fossilis*) under polyculture system in farmer's seasonal ponds;
- To assess the effect of stocking density on the water quality parameters of the cultured farmer's seasonal ponds; and
- To analyze the benefit cost ratio of the cultured technology.

CHAPTER 2



REVIEW OF LITERATURE

CHAPTER 2

LITERATURE REVIEW

Aquaculture is defined as the culture of aquatic organisms in favorable environment. Stocked the selected species in natural water bodies, fertilizers and feeds are applied to encourage the production. When the surrounding environment provides them sufficient nutrients and good water quality parameters fishes grow well. Sometimes, excessive feeding rate may cause the deterioration of water quality and affects on growth. Before commencement of an experiment, it is needed to get information on the previous related research work. However, the research works done in various parts of the world are reviewed below for clear and better understanding of the present investigation. Data on the polyculture system of shing in the northern region was very scanty. There were some works done in the past are highlighted here:

2.1 Stocking density

Rahman *et al.* (2016) evaluated an experiment to assess the effects of stocking density on growth and production of shing (*H. fossilis*) in ponds. They were tested three different stocking densities at 80, 160 and 240 fish dec^{-1} and designated as T_1, T_2 and T_3, respectively. They stated that the highest production was obtained in T_3 but individual growth performance of shing (*H. fossilis*) was highest in T_1.

Haque *et al.* (2015) carried out an experiment to assess the production and growth performances of carps in different stocking densities of polyculture. They were tested three treatments of different carps i.e. rohu (*Labeo rohita*), catla (*Catla catla*), mrigal (*Cirrhinus cirrhosus*) and silver carp (*Hypophthalmicthys molitrix*) at a stocking densities were 40, 80 and 160 individuals $dec.^{-1}$ in T_1, T_2 and T_3 respectively and

reported that the stocking density of 80 fingerlings dec^{-1} (T_2) was the most suitable to ensure highest production in polyculture.

Monir and Rahman (2015) conducted an experiment to assess the effect of stocking density on growth, survival and production of shing (*H. fossilis*) fingerlings under nursery ponds in Northern region of Bangladesh. Three stocking densities were tested for each, viz., 2 million fry ha^{-1} (T_1), 2.5 million fry ha^{-1} (T_2) and 3 million fry ha^{-1} (T_3) and reported that the growth performances and survival of *H. fossilis* fingerlings were significantly higher in T_1 than those obtained from T_2 and T_3.

Zahidah *et al.* (2015) revealed an experiment to assess the effect of density ratio on performance of nile tilapia and catfish in polyculture fish farming system. They determined the stocking density ratio of both the catfish and the nile tilapia include: A (75:75 fry m^{-2}), B (100:50 fry m^{-2}) and C (125:25 fry m^{-2}) and indicates the highest chance of growth rate and survival rate were found in treatment A.

Hossain *et al.* (2014) evaluated the effects of fry stocking densities on growth, survival rate and production of *H. molitrix*, *Cyprinus carpio* var. *specularis* and *L. rohita* in earthen ponds at Natore fish farm, Natore, Bangladesh. They were tested two treatments where mean individual stocking weight (g) of the fry of silver carp, mirror carp and rui were 0.10, 0.103 and 0.08 respectively under treatment-I and were 0.17, 0.142 and 0.11 under treatment-II and reported that the net fish growth, survival rate and production was better in treatment-II.

Rahman *et al.* (2014) studied an experiment to assess the culture potentials of stinging catfish shing (*H. fossilis*) under different stocking densities in Northern Region of Bangladesh. They were tested three stocking densities at 185000 ha^{-1} (T_1), 2,00,000 ha^{-1} (T_2) and 2,25,000 ha^{-1} (T_3) and reported that the highest production obtained in T_1.

Shoko *et al.* (2014) stated that the effect of stocking density on growth, production and economic benefits of mixed sex nile tilapia (*O. niloticus*) and African sharptooth catfish (*C. gariepinus*) in polyculture and monoculture. They were investigated the effect of stocking density on growth, yield and economic benefits of nile tilapia (*O. niloticus*) in monoculture and polyculture with African sharptooth catfish (*C. gariepinus*) and demonstrated that farmers were achieved the highest net yield and economic benefits by culturing *O. niloticus* and *C. gariepinus* in polyculture.

Hossain *et al.* (2013) evaluated an experiment to assess the study on present status of carp-SIS polyculture in Dinajpur district of Bangladesh. They were used pond size of three different sectors, small, medium and large according to decimals and revealed that the highest income was found in the large pond category.

Chattopadhyay *et al.* (2013) carried out an experiment to assess the effects of stocking density of *L. rohita* on survival, growth and production in cages. They were reared six stocking densities (5, 7.5, 10, 15, 20 and 25fish m^{-2}) each with 3 replicates and maximum production and profit was observed at the highest stocking density.

Asadujjaman *et al.* (2013) carried out an experiment on the effects of stocking density on growth performance and production of mola, *Amblypharyngodon mola*. They were tested three treatments with different stocking density of mola at 145000, 73000 and 36500 individual's ha^{-1} which designated as T_1, T_2 and T_3 and observed the highest net production at the stocking density applied in T_3.

Alim (2013) evaluated an experiment to assess the effects of stocking density on growth and production of monosex male tilapia (*O. niloticus*) in ponds during the period of two months. Three stocking densities used were 50, 100 and 150 fish dec^{-1} and designated as T_1, T_2 and T_3. The production was 1.40, 2.58 and 3.59 kg dec^{-1} in T_1, T_2 and T_3,

respectively. But the highest production of 3.59 kg dec^{-1} was obtained in T_3 with stocking of 150 fish dec^{-1} due to higher stocking density.

Siddiquee *et al.* (2012) conducted the impact of exotic carps in the polyculture with indigenous carps: competition for food. They were cultured together of indigenous carps such as catla (*C. catla*), rohu (*L. rohita*) and mrigal (*C. cirrhosus*) with exotic carps such as silver carp (*H. molitrix*), bighead carp (*Aristichthys nobilis*) and mirror carp (*C. carpio*) in a fish pond and observed the higher level of dietary overlap between rohu and silver carp followed by rohu and bighead carp and between catla and silver carp.

Jannat *et al.* (2012) carried out an experiment to assess the effects of stocking density on survival, growth and production of Thai climbing perch (*Anabas testudineus*) under fed ponds. They were investigated that three treatments and the stocking densities were 350, 400 and 550 individuals per decimal and revealed that the growth parameters were higher in ponds with lower stocking density than the ponds with higher stocking density.

Kohinoor *et al.* (2012) conducted an experiment to assess the effects of stocking density on growth and production performance of indigenous stinging catfish, *H. fossilis* (Bloch). They were tested three different stocking densities at 1,25,000 (T_1), 1,87,500 (T_2) and 2,50,000 ha^{-1} (T_3) and reported that the highest mean harvesting weight was found in T_1 but the best survival was found in T_1.

Chakraborty and Nur (2012) reported that the growth and yield performance of shing, *H. fossilis* and koi, *A. testudineus* in polyculture were assessed at a stocking density of 2,47,000 ha^{-1} and 3,70,500 ha^{-1} respectively and production 18,803 kg ha^{-1} to 10,042 kg ha^{-1} in different treatments.

Jahan (2012) was conducted an experiment to observe growth performance of Thai koi (*A. testudineus*) under different stocking densities in monoculture and polyculture system over a period of 110 days. The stocking densities were 2000 and 1000 fry dec^{-1} in T_1 (monoculture) and T_2 (polyculture). The best growth performance was recorded in T_2 (90.75g) than T_1 (66.55g) at lower stocking densities.

Dev (2009) conducted an experiment for 165 days to evaluate the growth and production of fishes at different species combinations in polyculture using *O. niloticus, L. rohita and H. molitrix.* Three stocking densities 268,288 and 312 fish dec^{-1} were given in T_1, T_2 and T_3 respectively. Among the three treatments the highest production was found in T_1 (7627.20 kg dec^{-1}) which were significantly higher than other treatments at lower stocking densities.

Jannat (2009) studied on the effects of stocking density on the growth, survival and production of Thai koi (*A. testudineus*) fed on formulated diet in farmer's ponds over a period of 90 days. Three different stocking densities were tested viz., 350, 400 and 550 fry dec^{-1} in T_1, T_2 and T_3 respectively. At the end of the study highest growth, survival and production was found in lower stocking densities.

Azad *et al.* (2004) described the polyculture of carp, tilapia and pangas using low cost inputs. They were evaluated three treatments in polyculture using tilapia (*O. niloticus*), pangas (*Pangasius hypophthalmus*), mrigal (*C. cirrhosus*) and silver carp (*H. molitrix*) at the stocking density was 100 fish dec^{-1} at different species compositions and reported that pangas showed the lowest growth performance and production in all treatments compare with other species.

Roy *et al.* (2003) analyzed the economics of carp SIS polyculture in rural farmer s pond. They tested three treatments consist of stocking the ponds with only carp's (T_1), carps

with mola (T_2) and carps with chela (T_3) and found higher benefit in carp-mola polyculture system.

Khan *et al.* (2003) evaluated an experiment to assess the marginal analysis of culture of stinging catfish (*H. fossilis*, Bloch): effect of different stocking densities in earthen Ponds. They were tested four different stocking densities were tested, namely treatments T_1 (40,000 individuals ha^{-1}), T_2 (60,000 individuals ha^{-1}), T_3 (80,000 individuals ha^{-1}) and T_4 (1, 00,000 individuals ha^{-1}) and reported that 60,000 individuals ha^{-1}stocking density (T_2) would be the best recommendation for farmers.

Jena *et al.* (2001) conducted an experiment to assess the comparative evaluation of growth, survival and production of carp species at different stocking densities under polyculture. They were conducted in six ponds at a stocking density ranging between 2000 and 6000 numbers $ha^{-1}$and observed that growth of all the species decreased with increase in stocking densities.

Kohinoor *et al.* (1998) designated the Effect of mola (*A. mola*) on the growth and production of carps in polyculture. They were studied the effect of introduction of mola (*A. mola*) in polyculture with rohu (*L. rohita*), catla (*C. catla*) and mirror carp (*C. carpio* var. *specularis*) in semi-intensive culture system and found to exert a negative impact on growth and production of carps.

Haque and Razzaque (1998) carried out a polyculture experiment maintaining the stocking density and ratio of rohu, catla, silver carp, mrigal, common carp and Thai shorpunti at the rate of 8000-10000 fish ha^{-1} in the ratio of 16:12:15:12:15:30 and reported that the yield found at 18-20 kg dec^{-1} in six months (4500-5000 kg of fish ha^{-1} in six months).

Kohinoor *et al.* (1998) described the growth and production performances of red tilapia and nile tilapia (*O. niloticus*, Lin.) under low-input culture system. They found that the gross fish production of 3218 and 3017 kg ha^{-1}were obtained from *O. niloticus* and red tilapia ponds respectively.

Charoentesprsit *et al.* (1997) was investigated an experiment on climbing perch. *A. testudineus* was stocked at 3 stocking densities 100, 200 and 300fish m^{-2} and fed on diets containing 20, 25 and 30% protein for 12 weeks. Fish stocked at a density of 200 $m^{-2}$and fed on 30% protein showed optimum specific growth rate, length gain, weight gain, protein efficiency ratio, feed cost, weight gain and survival rate.

Alam *et al.* (1995) conducted the effect of stocking density on the growth and survival of magur (*C. batrachus*, Linn.). The experiment was completed for period of 4 months, starting from 1st September to 31st December 1987 in the Fisheries Research Institute, Mymensingh, Bangladesh. The study was passed out according to 3x3 randomized block design. Three stocking density, viz., 2,500 ha^{-1}, 5,000 $ha^{-1}$and 10,000 ha^{-1} were employed. In the experiment it was exposed that decreased stocking density favors increased growth rate and vice versa. Growth rate stunted gradually in respect of length and weight. On the other hand, the stocking density had a significant influence upon length and weight ($P<0.01$) but survival percentage remain same.

Das *et al.* (1992) stated the growth of *C. batrachus* had an inverse relationship with the stocking density. They recommended that the lower stocking density showed the highest growth.

Haque and Barua (1989) were carried out an experiment on the effect of stocking density on larval rearing of *H. fossilis*. Five days old *H. fossilis* larvae were reared at four

stocking densities viz. 6, 12, 24 and 48 larvae L^{-1} of water for a period of four weeks. They observed that stocking density has significant ($P<0.05$) upon growth and survival.

Rouse *et al.* (1987) reported the effects of stocking size and density of tilapia on *Macrobrachium rosenbergii* in Polyculture. They were tested two treatments which one was monoculture of prawn and another was polyculture of prawn with tilapia and reported that survival was highest in polyculture ponds.

Mollah (1985) stated that the lower stocking density gave larger size and higher survival rate in *C. macrocephalus.*

Tarnchalanukit *et al.* (1983) was carried out an experiment on fry rearing of *C. batrachus* in circular concrete ponds with circulating system as average of 0.1g fry were stocked in six $15m^3$ ponds by supplying floating pelleted feed for 3 months. They pointed out that lower stocking density gave higher growth and medium gave higher survival.

Pathak (1978) conducted an experiment with *A. testudineus* in two cisterns for six months duration with 300 juveniles (average wt. 1.05g) in each. He reported total production of 8.12 and 7.81 kg respectively with 80% and 65% survival and average weight attained by individual fish was around 33.83 and 40.04 g in six months.

Backiel and Le Cren (1978) stated the stocking density as important parameters in fish culture operations.

INFS (1977) some of the freshwater fishes like shorpunti, koi, tengra, shing, magur etc. contain high amount of protein, vitamin, iron and minerals.

Powell (1972) described that higher stocking density has harmful effects on the fish growth, survival and production and an increase of food conversion ratio.

Le Cren (1965) demonstrated the growth of fish was dependent on the population density. He further reported that a direct relationship existed between food abundance and growth rate as between population density of the species and its growth rate tend to be inversely related.

2.2 Water quality parameter

Haque *et al.* (2015) carried out the physico-chemical parameters of water for a period of 3 months in nine experimental ponds in the field laboratory of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. They reported the ranges of physico-chemical parameters of water as temperature 31.61-32°C, transparency 30.69-34.11cm, pH 7.48-7.76, dissolved oxygen 5.33-7.56 mg L^{-1}.

Rahman *et al.* (2014) described the physico-chemical parameters at nine experimental mini ponds for a culture period of seven months in Freshwater Sub-station, Bangladesh Fisheries Research Institute, Saidpur, Nilphamari. They observed the ranges of physico-chemical as temperature 26.96-27.94°C, transparency 28.78-31.93 cm, pH 7.62-7.81, dissolved oxygen 4.34-4.89 mg L^{-1}, total alkalinity 103.07-115.93 mg L^{-1} and ammonia 0.08-0.12 mg L^{-1}.

Hossain *et al.* (2014) indicated the physico-chemical parameters of water at the Banbelgharia, the Government fish seed production farm, Natore for a period of 3 months in six experimental fish ponds. They reported the ranges of water temperature 26 to 34°C, transparency 28 to 41 cm, dissolved oxygen 2.45 to 5.5 mg L^{-1}, pH 7.0 to 8.5, total alkalinity 130 to 182 mg L^{-1} and ammonia nitrogen 0.12 to 0.3 mg L^{-1}.

Asadujjaman *et al.* (2013) conducted the physico-chemical parameters of water for a period of 90 days in nine similar sized ponds at the Fisheries Field Laboratory complex,

under the faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh. They indicated the ranges of temperature 26.57-26.97°C, transparency 44.38-45.62 cm, pH 8.22-8.47, dissolved oxygen 6.43-6.58 mg L^{-1}, total alkalinity 133.24-145.67 mg L^{-1} and ammonia 0.19-0.37 mg L^{-1}.

Kohinoor *et al.* (2012) observed the physico-chemical properties of nine farmer's ponds for a period of six months at Modhupur, Tarakanda and Mymensingh. They reported the ranges of physico-chemical parameters as temperature 24.60-30.30°C, transparency 20.14-26.00 cm, pH 7.08-8.70, dissolved Oxygen 4.23-5.32 mg L^{-1}, total alkalinity 102.00-123.00 mg L^{-1} and total ammonia 0.033-0.055 mg L^{-1}.

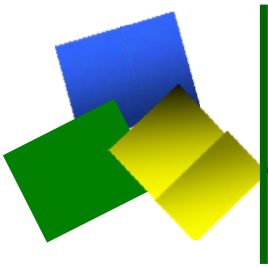
Chakraborty and Nur (2012) conducted the Physico-chemical parameters of the private rearing ponds of three Fish Farms in Sadar, Gouripur and Gaffargaon Upazilla, Mymensingh. They studied the range of temperature as 25.81-25.98°C, transparency 25.54-36.24 cm, pH 8.08-8.20, dissolved oxygen 4.05-4.4.72 mg L^{-1} and total alkalinity 153.23-160.60 mg L^{-1}.

Kohinoor *et al.* (1998) described the water quality parameters in six earthen ponds for a period of six months at Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. They carried out the ranges of water temperature 27.55-27.72°C, transparency 32.1-32.50 cm, pH 7.18-7.20, dissolved oxygen 4.20-4.45 mg L^{-1}, total alkalinity 104.81-114.54 mg L^{-1} and ammonia 0.14-0.15 mg L^{-1}.

Mollah and Haque (1978) conducted an experiment on physico-chemical properties of pond water in Bangladesh Agricultural University Campus, Mymensingh and recorded temperature of 26.0°C-32.44°C, pH of 7.19-7.44, DO of 1.19-7.74 mg L^{-1}.

Bhuiyan (1970) determined the low pH (<7.0) was not suitable for fish production. He also stated that the concentration of phosphate ranging from 0.2 to 0.4 mg L^{-1}, nitrate from 0.063 to 0.1 mg L^{-1} and dissolved oxygen from 5.0 to 7.0 mg L^{-1} was within good range for high production.

Michael (1969) carried out the quantity of DO present in the ponds was dependent on the various biological processes taking place in the medium. He also stated that the pH ranging between 7.3 and 8.4 was good for fish culture.



CHAPTER 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

This experiment was conducted to fulfill the objectives of this research. For this experiment, the study was carried out to determine the effects of stocking density on growth, survival and production of shing with polyculture system in grow out ponds.

3.2 Study area

The experiment was carried out in Saidpur and Dimla Upazila of Nilphamari district and Taragonj Upazila of Rangpur district.

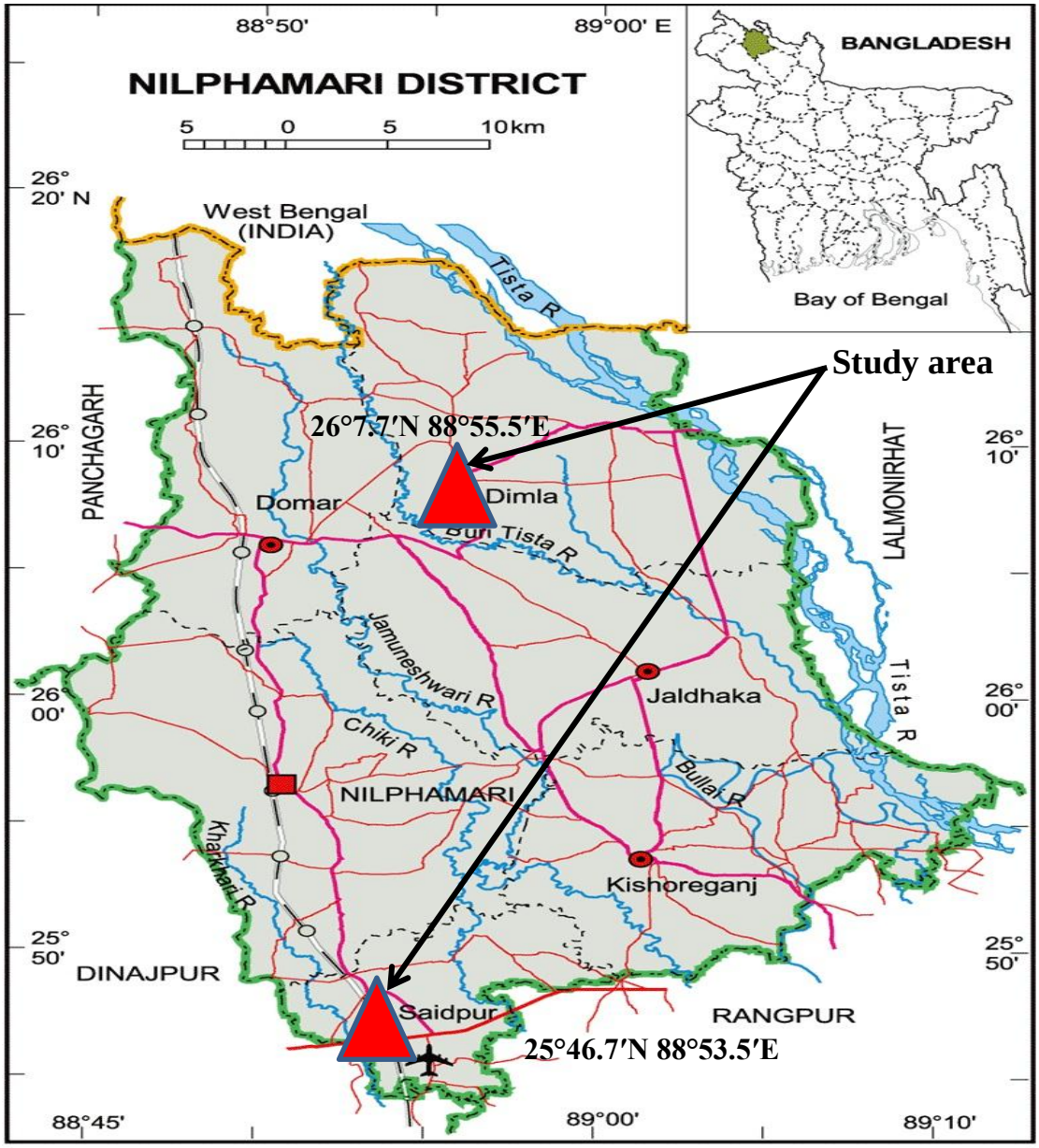


Figure 3.1 Map showing the study area of Dimla and Saidpur Upazila of Nilphamari District.

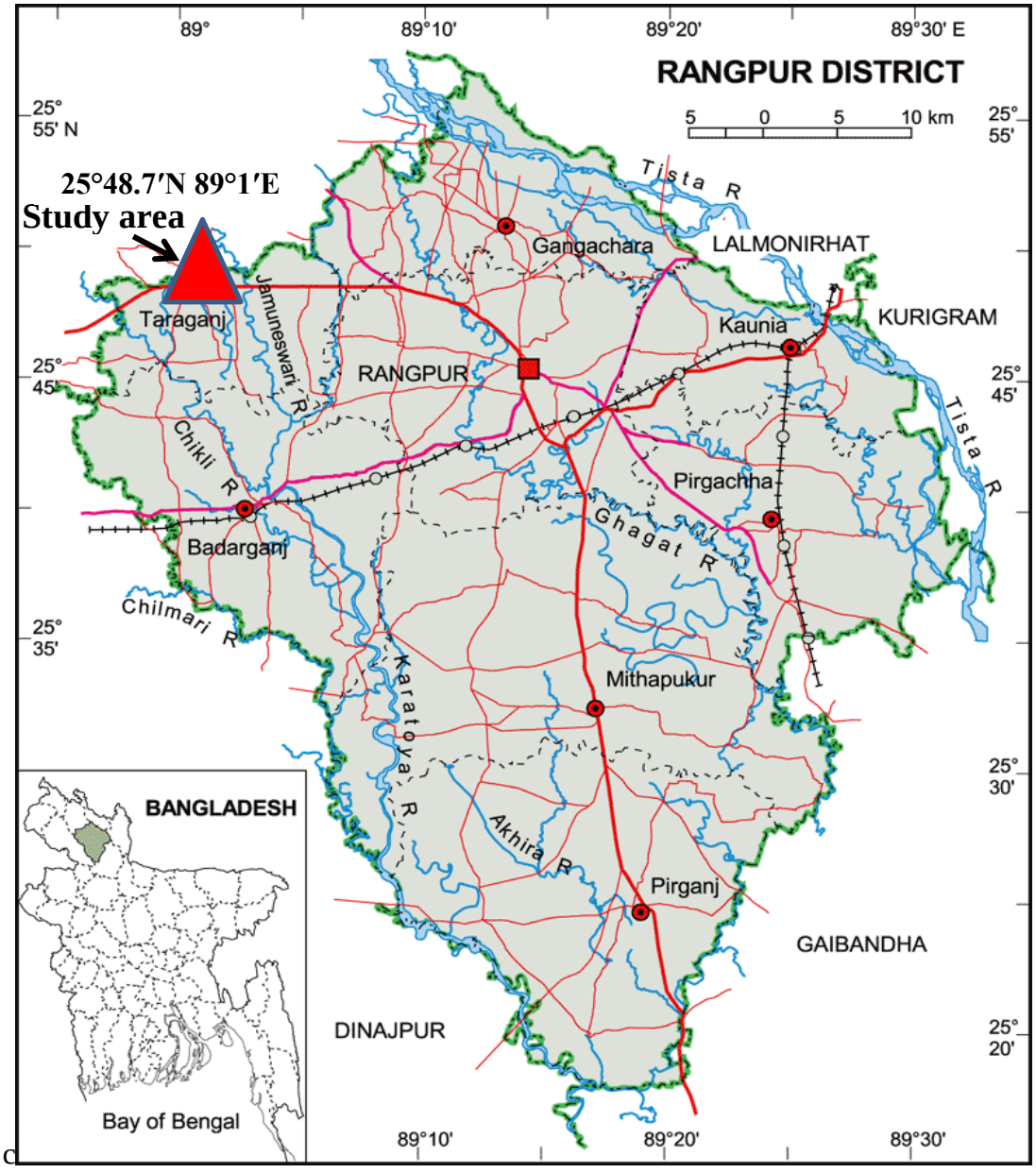


Figure 3.2 Map showing the study area of Taragonj Upazila of Rangpur District.

3.3 Experimental period

The study was conducted for a period of 150 days from 30 March to 30 August 2016.

3.4 Description of experimental units

Nine earthen seasonal ponds were selected at Saidpur and Dimla Upazila of Nilphamari district and Taragonj Upazila of Rangpur district for the experimental purpose. Rectangular and semi rectangular ponds were selected and the size and depth of the experimental ponds were around 10 decimal and 4 feet respectively.

In order to proper management and protect from poaching the ponds were selected near the house of the farmers. The experimental ponds were also situated a side where sunlight was available. The main source of water was the rainfall. In some cases shallow machine pump was used by a flexible plastic pipe if needed. The embankment was well protected and covered with grass which prevent from soil erosion.

3.5 Design of experiment

The experiment was conducted with three treatments (T_1, T_2 and T_3) each having three replications (R_1, R_2 and R_3). Three different stocking densities of shing fingerlings were stocked in the experimental ponds where the other species remain in constant densities.

The design of polyculture patterns are showing in Table 3.1.

Table 3.1 Polyculture of shing under different stocking densities in the farmer's ponds.

Treatments	Replication	Species	Stocking Density (Ind. dec^{-1})
T_1	R_1 R_2 R_3	shing + magur + GIFT + shorputi	500+50+10+5
T_2	R_1 R_2 R_3	shing + magur + GIFT + shorputi	600+50+10+5
T_3	R_1 R_2 R_3	shing + magur + GIFT + shorputi	700+50+10+5

3.6 Pond preparation

3.6.1 Removal of undesirable species and aquatic weeds

Before commencement of the experiment all ponds were renovated and removed of aquatic vegetation manually. The weeds of embankment of the experimental ponds were also removed manually. The dikes of the experimental ponds were also repaired. Undesirable fishes and other harmful aquatic organisms were removed by using rotenone at the rate of 25-35 g dec^{-1} ft^{-1} where necessary. Then the dead fishes and other aquatic organisms were removed by repeated netting.

3.6.2 Liming

After one week of rotenone application, ponds were prepared by applying lime at a rate of 1 kg dec^{-1}. Lime was mixed in water into an earthen pot and then spread homogenously all over the ponds.

3.6.3 Fertilization

After 4-5 days of liming, ponds were fertilized using cow-dung at a rate of 5-6 kg dec^{-1}, Urea 100 g dec^{-1} and TSP 75 g dec^{-1} at the initial stage of pond preparation. Before

application of TSP, it was needed to dissolve in plastic buckets with water for about 10 to 12 hours. Then the dissolved fertilizers were spread all over the ponds.

3.7 Experimental fish

The fingerlings of shing (*H. fossilis*) were selected for the experimental purposes. Shing fingerlings (average weight 1.5 g and length 5.35 cm) were brought from a private hatchery Ranirbandar, Dinajpur and transported to the BFRI sub-station using oxygenated plastic bags filled with freshwater. Prior to the commencement of the study, the shing fingerlings were acclimatized for 15 days in the pond of BFRI sub-station, Saidpur. Thereafter, fish with an average weight of 3 g and average length 8.22 were stocked at the densities of 500, 600 and 700 fingerlings dec^{-1} of pond which represented as T_1, T_2 and T_3 respectively and constant densities of 50, 10 and 5 fingerlings dec^{-1} of pond were maintained for magur, GIFT and shorputi respectively. Before the release of fingerlings in the experimental ponds, the containers were kept in surface water in the experimental ponds for about 30 minutes for temperature acclimatization. After 30 minutes, the fingerlings were released in the experimental ponds (Plate 3.1).

Plate 3.1 Stocking of fish in the experimental pond.

3.8 Feeding

After each sampling, the average weight of fishes was recorded for calculating the total amount of feed required for each experimental pond. During the culture period, the experimental fishes were fed with a commercial feed (Lily fish feed) containing 35.53% protein twice daily at 5-10% body weight of the experimental fish. Feed was distributed evenly all over the pond's surface two times daily at morning and evening.

3.9 Monitoring and data collection

For the evaluation of growth performances, the sampling was done in fifteen days intervals. In each replication, sampling was done randomly by using a ber net and weight was measured by using a digital balance to adjust the amount of feed to be given (Plate 3.2). During sampling, the length of the fishes was also recorded by using a steel scale. Throughout the experimental period, ponds were visited regularly in order to maintain the ponds as well as the health conditions of shing and other three species. Sampling was done very carefully to avoid the handling stress and mechanical injury.

Plate 3.2 Determination of weight (g) of shing during the study period.

3.10 Water quality assessment

3.10.1 Procedure of the study

During the study period, the water samples were collected from the experimental ponds between 9.00 to 11.30 AM. Water temperatures, transparency, pH, dissolved oxygen (DO), total alkalinity and total hardness were recorded fortnightly.

3.10.1.1 Temperature

By using a standard mercury thermometer the water temperature of each pond was recorded (Plate 3.3).

Plate 3.3 Determination of water temperature during the experimental period.

3.10.1.2 Transparency

The transparency of water was recorded from each pond by using a secchi disc (Plate 3.4).

Plate 3.4 Determination of transparency during the experimental period.

3.10.1.3 pH

The pH of water of the experimental ponds was measured during the culture period by using a digital pH meter (Elico-Li-120) (Plate 3.5).

Plate 3.5 Determination of pH during the experimental period.

3.10.1.4 Dissolved Oxygen

The dissolved oxygen of the experimental ponds was recorded by using a digital DO meter (YSL, Model 58, USA).

3.10.1.5 Total Alkalinity

The total alkalinity was measured by applying titrimetric method using bromophenol or blue indicator and HI: 3811-0 solution (alkalinity test kit HI 3811) as explained detailed in Appendix A.1.

3.10.1.6 Total Hardness

The total hardness was measured by applying EDTA and HI: 3812-0 solution (Hardness Test kit HI 3812) as explained in Appendix A.2 (Plate 3.6).

Plate 3.6 Determination of total hardness during the experimental period.

3.10.1.7 Ammonia

The amount of ammonia in the experimental ponds was measured using an ammonia test kit (Hanna, Romania) as described detailed in Appendix A.3.

3.11 Harvesting

Fishes were completely harvested after 150 days of culture period. Harvesting of fishes was done by complete dewatering of the ponds. After harvesting, the weight of all fishes was taken.

3.12 Growth performance and production of fish

At the end of harvesting the following growth performances and survival were evaluated by using the following equations:

(i) Growth

a) Weight gain

$$\text{Weight gain (g)} = \text{Final weight (g)} - \text{Initial weight (g)}$$

b) % Weight gain

$$\text{Percent weight gain (\%)} = \frac{\text{Final weight (g)} - \text{Initial weight (g)}}{\text{Initial weight (g)}} \times 100$$

c) Average daily gain (ADG % day^{-1})

$$\text{Average daily gain (\%/day)} = \frac{\text{mean final weight} - \text{mean initial weight}}{\text{days}} \times 100$$

d) Specific growth rate (SGR % day^{-1})

The SGR is the momentary change in weight of fish calculated as the percent increase in body weight per day over a given time interval and is written as:

$$\text{SGR (\% /day)} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1} \times 100$$

Where,

W_1= the initial live body weight (g) at time T_1 (day).

W_2= the final live body weight (g) at time T_2 (day).

e) Survival rate (%)

Survival of fish was calculated by using the following formula:

$$\text{Survival rate (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

3.13 Production of fishes

Production is calculated by the following formula:

Net production= No. of fish caught × average final weight (g).

3.14 Proximate composition analysis

Sampling procedure

For the determination of whole body proximate composition and body indices, three fish were randomly selected from each replicate pond (nine fish per treatment) and brought to the laboratory. The selected fish were then killed by keeping them in a freezer. After being killed, fish were weighted immediately and then dissected. Whole fish body, liver, intraperitoneal fat, intestine and viscera weight were recorded individually (nine fish per treatment) at the end of the culture period for the determination of the body indices parameters such as hepatosomatic index (HSI), intraperitoneal fat (IPF) and viscerasomatic index (VSI).

Whole body proximate composition of shing was evaluated by using the standard protocols mentioned by the Association of Official Analytical Chemists (AOAC, 1997).

3.14.1 Moisture

The moisture content of the respective samples was determined by drying the samples at 105°C for 24 hours in the Hot Air Oven until constant weight was obtained as explained

in detail in Appendix B.1. The percent moisture content was calculated by using the following formula:

$$\%\ \text{of Moisture} = \frac{\text{E}}{\text{C}} \times 100$$

Where,

E= Weight of moisture.

C= Weight of sample.

Then the moisture free samples were used to determine the crude protein, lipid and ash content.

3.14.2 Crude protein

Crude protein content of the samples was analyzed by using the Kjeldhal method by using kjeldhal apparatus. By digesting the sample with concentrated sulphuric acid (H_2SO_4) total nitrogen content was determined, in presence of digestion mixture into boric acid as explained in detail in Appendix B.2.

Then the total nitrogen value was calculated by the following formula:

$$\text{Nitrogen}\ \% = \frac{\text{ml. of titrant used} \times \text{ normality of titrant} \times \text{ milli equivalent weight of Nitrogen}}{\text{Weight of the sample(g)}} \times 100$$

The amount of crude protein was then calculated by multiplying the % of total nitrogen with the Protein conversion factor 6.25, which is generally used in calculating the animal protein content.

3.14.3 Lipid

Crude lipid content of the samples was determined by extracting the lipid from the samples by homogenizing it in 60 ml of chloroform and methanol solution in a ratio of 2:1 (v/v) (Folch *et al.*, 1957) and therefore evaporation of the solvent in the oven at 80^0 C

as explained in detail in Appendix B.3. Then crude lipid content was calculated by the following formula:

$$\text{Lipid content } \% = \frac{\text{Weight of beaker with lipid (g)} - \text{Weight of empty beaker (g)}}{\text{Weight of sample (g)}} \times 100$$

3.14.4 Ash

The crude ash content of the samples was quantified by using muffle furnace, desiccator and an electronic balance. The sample was taken in a porcelain crucible and weighed, then the ash content was determined by keeping the crucible in a muffle furnace for about six hours at 550°C. Then the samples were needed to cool by using desiccator. Then the remaining materials of each sample was taken as ash as explained detail in Appendix B.4.

Ash content of the whole fish body was determined by using the following formula:

$$\% \text{ of Ash } = \frac{\text{Weight of crucible with ash (g)} - \text{weight of empty crucible (g)}}{\text{Weight of sample (g)}} \times 100$$

3.15 Determination of body indices

At the end of the present experiment, body indices of shing such as hepatosomatic index (HSI), intraperitoneal fat (IPF) and viscerasomatic index (VSI) were determined by using the following formula:

$$\text{Hepatosomatic index (HSI\%)} = \frac{\text{Liver weight (g)}}{\text{Body weight (g)}} \times 100$$

$$\text{Intraperitoneal fat (IPF\%)} = \frac{\text{Intraperitoneal fat weight (g)}}{\text{Body weight (g)}} \times 100$$

$$\text{Viscerosomatic index (VSI\%)} = \frac{\text{Viscera weight (g)}}{\text{Body weight gain (g)}} \times 100$$

3.16 Economic analysis

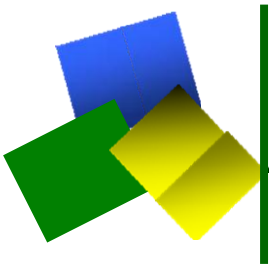
At the end of the experiment economic analysis was determined by using the following formula:

Gross margin (BDT treatment^{-1}) = Gross return - variable cost

BCR = Gross return/variable cost (BDT)

3.17 Data analysis

All data were tested using one-way analysis of variance (ANOVA). Significant results ($P< 0.05$) were further tested using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) to identify significant difference between means. The data were expressed as average ± SE and statistical analysis was performed using SPSS version 22 and Microsoft Office EXCEL for window.



CHAPTER 4

RESULTS

CHAPTER 4

RESULTS

4.1 Water quality parameters

The water quality parameters over the polyculture system of shing with magur, tilapia and shorputi of all treatments are presented in Table 4.1. Generally, there was no significant variation in case of temperature, transparency, pH and dissolved oxygen but the significant variation was observed in case of total alkalinity, hardness and ammonia among treatments.

In the present experiment the recorded water temperature was in treatments T_1, T_2 and T_3 as 27.79±0.14, 28.35±0.10 and 27.94±0.13 respectively (Table 4.1). However there was no significant ($P>0.05$) variation among the treatments, while a comparatively highest temperature was observed in the treatment of T_2. Similarly a non-significantly ($P>0.05$) highest transparency was noted in treatments of T_2 (27.62±0.42) compared to T_1 (27.41±0.27) and T_3 (27.55±0.39).

The pH values in all the treatments were not varied significantly (*P>0.05*), however a comparatively lower level of pH was observed in the treatment of T_1 (7.31). In different treatments the pH was observed as 7.31±0.04, 7.49±0.05 and 7.46±0.02 in T_1, T_2 and T_3 respectively as showed in Table 4.1. Similar result was also observed in the case of dissolved oxygen content (mg L^{-1}) in the experimental ponds that was recorded as 5.64±0.07, 5.49±0.11 and 5.77±0.04 in treatments T_1, T_2 and T_3 respectively (Table 4.1).

Generally an increasing trend was observed in the case of total alkalinity, hardness and ammonia content with the increasing of stocking density. The total alkalinity in different treatments of T_1, T_2 and T_3 were recorded as 99.54±0.86, 102.80±0.34 and 105.86±1.21

mg L^{-1} respectively (Table 4.1). Significantly highest ($P<0.05$) total alkalinity was take place in treatment T_3 (105.86±1.21) compared to T_1 (99.54±0.86) and T_2 (102.80±0.34) but the treatments T_1 and T_2 did not show any significant variation. In the case of total hardness, similar result was also observed. The hardness was noted as 39.53±1.01, 48.83±1.70 and 69.20±1.13 mg L^{-1} in treatments T_1, T_2 and T_3 respectively (Table 4.1). In all treatments, hardness were significantly ($P<0.05$) differed. Significantly highest value was noted in T_3 (69.20±1.13) and lowest value was recorded in T_1 (39.53±1.01).

In the entire experiments, ammonia (unionized) was recorded in three treatments T_1, T_2 and T_3 as 0.16±0.01, 0.20±0.01 and 0.24±0.03 mg L^{-1} respectively (Table 4.1). Comparatively lower amount of ammonia was found in T_1 than that of T_2 and T_3. Although the values of ammonia in T_2 (0.20 mg L^{-1}) and T_3 (0.24 mg L^{-1}) showed higher amount compared to T_1, however T_2 and T_3 did not vary significantly ($P>0.05$).

Table 4.1 Water Quality Parameters observed in different treatments of shing (*H. fossilis*) polyculture of the experiment over 150 days culture periods in ponds

Parameters	Treatments		
	T_1	T_2	T_3
Temperature (°C)	27.79±0.14[a]	28.35±0.10[a]	27.94±0.13[a]
	(27.62-28.08)	(28.23-28.58)	(27.67-28.12)
Transparency (cm)	27.41±0.27[a]	27.62±0.42[a]	27.55±0.39[a]
	(26.87-27.78)	(26.95-28.40)	(26.90-28.26)
pH	7.31±0.04[a]	7.49±0.05[a]	7.46±0.02[a]
	(7.22-7.36)	(7.38-7.57)	(7.41-7.48)
Dissolved Oxygen (mg L^{-1})	5.64±0.07[a]	5.49±0.11[a]	5.77±0.04[a]
	(5.51-5.78)	(5.30-5.70)	(5.68-5.85)
Alkalinity (mg L^{-1})	99.54±0.86[a]	102.80±0.34[ab]	105.86±1.21[c]
	(98.30-101.20)	(102.20-103.40)	(104.10-108.20)
Hardness (mg L^{-1})	39.53±1.01[a]	48.83±1.70[b]	69.20±1.13[c]
	(37.80-41.30)	(45.60-51.40)	(67.40-71.30)
Ammonia (mg L^{-1})	0.16±0.01[a]	0.20±0.01[ab]	0.24±0.03[b]
	(0.15-0.18)	(0.19-0.21)	(0.19-0.30)

Data presented as mean±SE. Data with different superscripts in the same row indicate significant ($P< 0.05$) differences

4.2 Growth performance

The growth performances of the polyculture of shing with magur, tilapia and shorputi under different stocking densities were recorded after 150 days rearing is given in Table 4.2. The growth of shing in ponds was investigated and the result showed that the growth rates were varied significantly according to their stocking densities.

Although, there is no significant difference in initial weight and initial length of fishes in different treatments but the final weight of *H. fossilis* were varied significantly ($P<0.05$). Final weight of *H. fossilis* was obtained as 65.11±0.64, 58.00±1.15 and 48.00±0.57 g in T_1, T_2 and T_3 respectively (Table 4. 2). Final weight was obtained highest in T_1 where the stocking density was lowest and lowest in T_3 where the stocking density was highest. Final weight of *H. fossilis* in T_1 (65.11 g) was varied significantly ($P<0.05$) compared with the remaining two treatments T_2 (58.00 g) and T_3 (48.00 g).

In the current experiment, final length of *H. fossilis* was obtained in different treatments in T_1, T_2 and T_3 as 21.05±0.02, 20.58±0.04 and 19.05±0.37 cm respectively (Table 4.2). Therefore, the results revealed that the higher final length was noted at the lower stocking densities. Final length in T_1 was significantly ($P<0.05$) higher than T_3, while T_2 was not differed significantly ($P>0.05$) from T_1.

Weight gain of *H. fossilis* in three treatments was recorded as 62.15±0.64, 55.01±1.14 and 45.00±0.57 g in T_1, T_2 and T_3 respectively (Table 4.2). Weight gain of *H. fossilis* in T_1 was varied significantly ($P<0.05$) compared to the remaining two treatments T_2 and T_3. Similar results were noted in the case of % weight gain and this was recorded as 2135.46±5.05, 1839.60±34.87 and 1500.00±19.22 in treatments T_1, T_2 and T_3 respectively (Table 4.2). The % weight gain of *H. fossilis* in T_1 (2135.46) was highest where the lowest stocking density take places. Significant variation ($P<0.05$) were

observed among the three treatments. In the case of average daily gain (ADG), a decreasing trend was observed in different treatments T_1 (0.41±0.01), T_2 (0.36±0.02) and T_3 (0.29±0.01) with increasing the stocking density and significant ($P<0.05$) variation were noted among the three treatments.

In the current study, the SGR value of shing was recorded as 2.04±0.01, 1.97±0.01 and 1.84±0.01 in different treatments T_1, T_2 and T_3 respectively (Table 4.2). The result showed that the value of SGR was decreased significantly while the stocking density was increased. SGR in T_1 (2.04) was significantly ($P<0.05$) higher than T_2 (1.97) and T_3 (1.84).

During the investigation of the present experiment, survival of shing was observed as 77.93±0.94, 76.12±0.36 and 72.71±0.08 in T_1, T_2 and T_3 respectively (Table 4.2). The result showed that the highest survival was observed in T_1 (77.93%) and the lowest survival was recorded in T_3 (72.71%). Significantly highest ($P<0.05$) survival rate of *H. fossilis* was noted in the T_1 (77.93%) compared to T_2 (76.12%) and T_3 (72.71%) but there was no significant differences between T_2 and T_3.

At the end of the experiment, the mean production of *H. fossilis* in different treatments was recorded as 25.38±0.55, 26.50±0.65 and 24.43±0.66 kg dec^{-1} in T_1, T_2 and T_3 respectively (Table 4.2). The highest production of *H. fossilis* was found in T_2 (26.50 kg dec^{-1}) which did not differed significantly ($P>0.05$) when compared with T_1 (25.38 kg dec^{-1}).

Table 4.2 Growth performances, feed utilization parameters and production observed in different treatments of shing (*H. fossilis*) polyculture of the experiment over 150 days culture periods in ponds.

Parameters	Treatments		
	T_1	T_2	T_3
Initial Weight (g)	2.95±0.01[a]	2.99±0.01[a]	3.00±0.00[a]
Final Weight (g)	65.11±0.64[c]	58.00±1.15[b]	48.00±0.57[a]
Initial Length (cm)	8.07±0.04[a]	8.22±0.01[a]	8.23±0.01[a]
Final Length (cm)	21.05±0.02[b]	20.58±0.04[b]	19.05±0.37[a]
Weight gain (g)	62.15±0.64[c]	55.01±1.14[b]	45.00±0.57[a]
Percent Weight gain (%)	2135.46±5.05[c]	1839.60±34.87[b]	1500.00±19.22[a]
Average daily gain(% day^{-1})	0.41±0.01[c]	0.36±0.02[b]	0.29±0.01[a]
Specific Growth Rate (SGR) (% day^{-1})	2.04±0.01[c]	1.97±0.01[b]	1.84±0.01[a]
Survival (%)	77.93±0.94[b]	76.12±0.36[a]	72.71±0.08[a]
Production of shing (kg dec^{-1})	25.38±0.55[ab]	26.50±0.65[b]	24.43±0.26[a]
Production of shing (kg ha^{-1})	6274.69±133.41[ab]	6547±161.92[b]	6036.65±65.62[a]
Total Production (kg ha^{-1})	7594.20±136.27[b]	7811.63±154.95[b]	6995.38±72.76[a]

Data presented as mean ± SE, obtained three replicate ponds (n=3); Data with different superscripts in the same row indicate significant differences *(P*< 0.05).

4.3 Proximate composition of shing (*H. fossilis*)

The whole body proximate composition of shing after 150 days of rearing is presented in Table 4.3. With the exception of protein content, no significant ($P>0.05$) variation was observed in the case of the whole body moisture, lipid and ash contents. A comparatively higher amount of moisture, protein and lipid were noted in the treatments of T_1 and lower content were observed in the treatments of T_3. In the case of ash content a relatively higher amount was observed in the treatments of T_2 while a lower amount was found in the treatments of T_1.

Table 4.3 Whole body proximate composition of shing (*H. fossilis*) in the polyculture system for a period of 150 days

Parameters	Treatments		
	T_1	**T_2**	**T_3**
Moisture (%)	71.05±0.38[a]	70.24±0.42[a]	69.30±0.82[a]
Protein (%)	17.29±0.33[b]	16.33±0.57[ab]	15.02±0.56[a]
Lipid (%)	5.88±0.34[a]	5.32±0.37[a]	5.28±0.64[a]
Ash (%)	5.70±0.63[a]	7.05±0.62[a]	6.57±0.58[a]

Data presented as mean±SE, in three replicate groups, (n=3). Data with different superscripts in the same row indicate significant ($P<0.05$) differences

4.4 Body indices of shing (*H. fossilis*)

At the end of the experiment, the body indices of shing after 150 days feeding are summarized in Table 4.4. Among all the body indices only intraperitoneal fat (IPF) showed an increasing trend with the increasing of stocking density. A non-significantly highest HSI was found in the ponds where lowest number of shing was stocked compared to another two treatments that were stocked at higher stocking density.

A significantly lower amount of IPF was observed in the fish that were stocked at a lowest stocking density (T_1) compared to those fishes that were stocked at highest stocking density. Unlike IPF, a significantly highest viscerosomatic index (VSI) was found in the treatment T_1 compared to T_2 and T_3.

Table 4.4 Body indices of shing (*H. fossilis)* in different treatments of the experiment over 150 days culture periods in ponds

Parameters	Treatments		
	T_1	**T_2**	**T_3**
Hepatosomatic Index (%)	1.15±0.15[a]	0.92±0.08[a]	1.00±0.19[a]
Intraperitonial Fat (%)	0.53±0.01[a]	0.74±0.08[ab]	0.87±0.06[b]
Viscerosomatic Index (%)	5.32±0.72[b]	3.48±0.0.18[a]	3.76±0.29[ab]

Data presented as mean±SE, (n=9; three fish from each replicate pond); Data with different superscripts in the same row indicate significant (P<0.05) differences.

4.5 Economic analysis

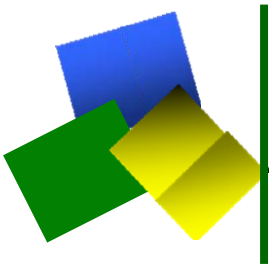
A simple Benefit cost ratio (BCR) was implemented to determine the profitability that had been achieved from these three types of culture systems. The expenditure in three different treatments were varied significantly (P<0.05) among themselves. The combined production of the fishes as observed in T_1, T_2, T_3 were 30.71±0.56, 31.48±0.51 and 28.31±0.29 kg dec^{-1} respectively. The production of fish was higher in T_2 but did not vary significantly with T_1. However the lowest production costs (BDT dec^{-1}) was recorded in T_1 (5610.92±10.09) followed by T_2 (6229.64±49.78) and T_3 (6330.05±74.06) (Table 4.5). Consistently higher net profit (BDT dec^{-1}) was observed in T_1 (3842.67±195.86) over T_2 (3514.58±149.85) and T_3 (2512.73±21.83) together with

significantly (P<0.05) higher BCR were recorded in T_1 (1.68±0.03) compared to T_2 (1.56±0.01) and T_3 (1.39±0.00) (Table 4.5).

Table 4.5 Benefit and cost analysis of shing polyculture (*H. fossilis* dec^{-1}) for culture period of 150 days

Items wise expenditures/ operational costs	**Treatments**		
	T_1	**T_2**	**T_3**
Pond preparation (BDT dec^{-1})	300	300	300
Price of fry (BDT dec^{-1})	1215	1415	1615
Lime, fertilizer, Cow dung (BDT dec^{-1})	50	50	50
Feed costs (BDT dec^{-1})	4045.92	4464.64	4365.05
Gross variable cost (BDT dec^{-1})	5610.92±10.09^{a}	6229.64±49.78^{b}	6330.05±74.06^{b}
Incomes and outputs			
Total production (kg dec^{-1})	30.71±0.56^{b}	31.48±0.51^{b}	28.31±0.29^{a}
Gross return (BDT dec^{-1})	9453.35±186.50^{b}	9744.24±199.54^{b}	8842.88±95.81^{a}
Gross margin (BDT dec^{-1})	3842.67±195.86^{b}	3514.58±149.85^{b}	2512.73±21.83^{a}
BCR	1.68±0.03^{c}	1.56±0.01^{b}	1.39±0.00^{a}

Data presented as mean±SE, (n=9; three fish from each replicate pond); Data with different superscripts in the same row indicate significant (P<0.05) differences.



CHAPTER 5

DISCUSSION

CHAPTER 5

DISCUSSION

The water quality parameters play an important role on the culture of fish and other organisms. Growth, feed efficacy and feed consumption of fish are normally governed by a few environmental factors (Brett, 1979). On the maintenance of a healthy aquatic environment and production of sufficient fish food organisms, environmental parameters exert an immense influence. For optimal fish growth and survival, good water quality is undoubtedly a prerequisite. The water quality parameters measured over the entire period of the study and were found to be more or less within the acceptable ranges for fish culture (Siddiquee *et al*., 2012).

Temperature is important parameters, which play a great role in respect of fish production. In the current experiment, the average water temperature was recorded as 27.79±0.14, 28.35±0.10 and 27.94±0.13°C in T_1, T_2 and T_3 respectively. Among the three treatments, there was no significant difference of temperature but those values were suitable for fish culture. From an experiment, Akhteruzzaman (1988) found water temperature 25.5°C to 30.0°C is favorable for fish culture. Similar results were also observed by Rahman *et al.* (1982), Patra (1993), Kohinoor *et al.* (1998) and Wahab *et al*. (2003). Haque (2014) who measured water temperature in ponds of BAU Campus, Mymensingh and found to vary from 24 to 32˚C. So in these cases, temperature ranges is suitable for fish culture.

In the present experiment, the average transparency level was observed in different treatments T_1, T_2 and T_3 as 27.41±0.27, 27.62±0.42 and 27.55±0.39 cm respectively. Water transparency ranges were observed as 26.87-28.40 cm in different treatments without having any significant difference, which was within the range of 26.75 to 30.44

cm as recorded by Kohinoor *et al.* (2016). The present experiment was also relevant with Hossain (2009), Ahmed *et al.* (2009). Therefore it can be said that the water transparency of the experimental ponds observed in the current study were favorable for fish culture.

pH is another most important factor in aquaculture production which also treated as the productivity index of a water body. Production of a water body was hampered, when abrupt changes of pH take places. The pH of the different treatments was found to be slightly alkaline and highest pH were observed as 7.49±0.05 in T_2 and lowest as 7.31±0.04 in T_1. Roy *et al.* (2002) obtained a pH range 7.03 to 9.03 in fish ponds located in Trishal, Mymensingh. The observed pH values of water ranging from 7.3 to 9.0 indicated that the experimental ponds were suitable for fish culture (Boyd, 1982). Ahmed *et al.* (2012) found pH range from 6.5 to 8.5 for health condition of farmed tilapia. Kohinoor *et al.* (2012) recorded pH range 7.08-7.15 for indigenous stinging catfish. The pH values observed in the current study were suitable ranges for shing culture. On the basis of pH value, it can be concluded that the experimental ponds were productive for fish culture.

Fish culture was successful, when the careful management of optimum level of dissolved oxygen takes places. In the current experiment, the average level of dissolved oxygen was obtained in different treatments T_1, T_2 and T_3 as 5.64±0.07, 5.49±0.11 and za5.77±0.04 mg L^{-1} respectively. DoF (1996) reported that the range of dissolved oxygen content for fish culture should be 5.0-8.0 mg L^{-1}. Among the three treatments, the dissolved oxygen level was fine and not varied significantly because of continuous water flow. From an experiment, Rahman (2000) and Maghna (2012) who measured dissolved oxygen (mg L^{-1}) in ponds of BAU Campus, Mymensingh and found to vary from 2.2 to 8.z8 mg L^{-1}, 3.40 to 8.79 mg L^{-1}, 7.5 to 7.6 mg L^{-1} and 4.8 to 5.4 mg L^{-1} respectively. Ahmed *et al.* (2012) measured dissolved oxygen ranged from 6 to 8.5 mg L^{-1} for the

culture of farmed tilapia. Fluctuation of dissolved oxygen might be attributed to photosynthetic activity and variation in the rate of oxygen consumption by fish and other aquatic organisms (Boyd, 1982). However, the level of dissolved oxygen in all the three treatments was acceptable ranges, impressive and very much productive for fish culture.

Total alkalinity values were depends on the season, location, nature of bottom deposits, plankton population etc. (Rahman *et al.*, 2014). Total alkalinity was observed in different treatments T_1, T_2 and T_3 as 99.54±0.86, 102.80±0.34 and 105.86±1.21 mg L^{-1} respectively. Total alkalinity shows the significance difference among the three treatments. Boyd (1982) advocated that the total alkalinity should be more than 20 mg L^{-1} in fertilized ponds as production increases with the increase in total alkalinity. From another study, Alikunhi (1957) reported that the total alkalinity more than 100 ppm should be present in highly productive water bodies. The variations of total alkalinity in all the treatments were within the productive range for aquaculture ponds (Wahab *et al.*, 1995; Kohinoor *et al.*, 1998).

In the current study, ammonia of the different treatments was reported as 0.16±0.01, 0.20±0.01 and 0.24±0.03 mg L^{-1} in T_1, T_2 and T_3 respectively. Highest content of ammonia were found in T_3 (0.24 mg L^{-1}) due to high stocking density and release of more amount of fecal materials in the ponds and lowest amount were found in T_1 (0.16 mg L^{-1}) due to low stocking density and less amount of fecal material were released in the ponds compared to T_2 and T_3. From an experiment, Kohinoor *et al.* (2001) found the ammonia-nitrogen ranged from 0.01-1.55 mg L^{-1} in monoculture system of small indigenous species. Wahid *et al.* (1997), Kohinoor *et al.* (1998), Paul (1998) and Kohinoor (2000) recorded that the amount of ammonia content in the ponds of BAU campus, Mymensingh were ranged from 0.01 to 0.99 mg L^{-1}. Thus it might be concluded that the ammonia content in the experimental ponds were acceptable for fish culture.

The effect of stocking density on growth and survival and production of *H. fossilis* was conducted and observed that the growth performance in earthen ponds varied on the different stocking densities. T_1 showed significantly highest growth ($P<0.05$) than those of T_2 and T_3. Although all the experimental fish were fed with the same diet at an equal ration, the result showed that the individual's growth rate of T_1 was the highest. This is might be because of relatively less number of similar size fish stocked in a pond could get more space and less competition for food and dissolved oxygen content.

In the present study, weight gain of shing (*H. fossilis*) was investigated. During the harvesting period, the highest weight gains of shing was observed in the lowest stocking density of T_1 (62.15g) and the lowest in the highest stocking density of T_3 (45g). Although same feed at a same ratio was applied in all the three treatments the results were varied significantly. These phenomenon indicated that lower stocking density reduces competition among the fishes which influenced them to take feed properly and it might be absent in the treatments with higher stocking densities. The present results coincide with the findings of Narejo *et al.* (2005) who achieved best growth at lower stocking densities shing farming. From a research, Kawamoto *et al.* (1957) reported the best growth at lower stocking densities in shing farming. Mollah (1985) reported that the lower density gave higher size and higher survival rate in *C. macrocephalus*. Therefore, it can be concluded that the higher weight gain take places at lower stocking densities.

Stocking density is an important parameter which directly affects the growth of fish and its production (Backiel and Le Cren, 1978). The highest value of percent weight gain was found inT_1 (2153.46) whereas the lowest was found in T_3 (1500). The results indicated that the percent weight gain varied with different stocking densities which coincides with the findings of Narejo *et al.* (2005) and Rahim (2010). From this context,

it can be said that, stocking density is known to be one of the important parameters in fish culture.

Specific growth rate of shing in different treatment T_1, T_2 and T_3 were found as 2.04±0.01, 1.97±0.01 and 1.84±0.0 respectively and also highest and lowest SGR value was observed in T_1 (2.04) and T_3 (1.84) respectively. The harmful effects of stocking density stated by Powel (1972), who observed less growth, survival rate at higher stocking densities. At higher stocking densities, presence of abundant feed substance could produce a competitive interaction among the larvae causing a stressful situation (Houde, 1975). A similar experience was observed by Lakshmanan *et al.* (1971) and Jena *et al.* (1998) while working with carps and other fish species. They obtained the highest values of specific growth rate at lowest stocking densities.

In the present experiment, the highest survivability of *H. fossilis* was recorded in T_1 (77.93%) and the lowest survivability was in T_3 (72.71%). From a research, Mollah (1985) reported that the lower stocking density resulted better growth and higher survival rate in *C. macrocephalus*. Survival was found to be negatively influenced by stocking densities. The reason for reduced survival rate due to higher stocking density of individuals as well as competition for natural food and space in the water area of pond which is supported by Tripathi *et al.* (1979), Haque *et al.* (1994) and Chakraborty *et al.* (2005).

At the end of the experiment, higher production of *H. fossilis* was obtained from T_2 (26.50 kg dec^{-1}) compared to T_1 (25.38 kg dec^{-1}) and T_3 (24.43 kg dec^{-1}) but there were no significant ($P>0.05$) difference between T_2 and T_1. It might be because of relatively higher numbers of fingerling were stocked in T_2 compared to those of T_1 but the highest individual growth was obtained in T_1. Therefore, the observed poor growth at higher stocking densities might be due to the effect of limiting space, stress initiated by excess

supplementary feeds, bad environmental conditions and lower amount of natural food in the culture system. The present result agreed with the findings of Mollah (1985), Narejo *et al.* (2005) and Siddik and Khan (2007).

The present study is the first time attempt to demonstrate the effect of stocking density on the proximate composition of fish. The result revealed that there is only a significant variation was found in the case of whole body protein content of fish. The result also represented that the protein and lipid content (as % wet weight basis) was lowest in the highest stocking density. Higher protein (17.29%) and lipid content (5.88%) and lower ash content (5.70 %) was found in the treatment of T_1 where the stocking density was the lowest.

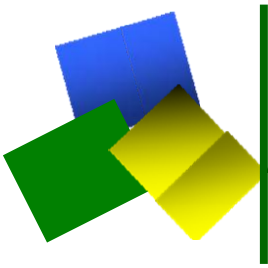
There is lack of available information on the effects of stocking density on body indices of fish. The present study is the first time to estimate the body indices on the basis of stocking density. With the exception of hepatosomatic index, intraperitoneal fat and viscerosomatic index showed a significant variation. Higher content of hepatosomatic index (1.15%) and viscerosomatic index (5.32%) and lower intraperitoneal fat (0.53%) content were found in the treatment of T_1 where the stocking density was the lowest. The increased protein content in the whole body and decreased fat content in the peritoneum might be a reason of increasing growth performances of shing in the lowest stocking density of T_1.

In the present experiment, the economic analysis of the culture systems was conducted in order to evaluate the economic profit under low input management approach. Although the expenditures in the treatment of T_2 and T_3 did not vary significantly ($P>0.05$), the lowest production costs (BDT dec^{-1}) was observed in T_1 (5610.92) followed by the remaining two treatments. However, significantly higher net profit (BDT dec^{-1}) was

found in T_1 (3842.67) followed by T_2 (3514.58) and T_3 (2512.73) which might be due to the lower stocking density of *H. fossilis* and highest individual weight of those *H. fossilis* that were found in T_1 then others treatments generated from 150 days culture period. From a study, Siddik and Khan (2007) recorded the cost and benefit of Monosex tilapia (*O. niloticus*) in monoculture system and got the net benefit of BDT 69,277.32 ha^{-1} from 6 months where fish were fed formulated feed. Kohinoor *et al.* (1993) observed that monoculture of Raj punti (*P. gonionutus*) gave a net benefit BDT 68,135 to 75,028 ha^{-1} from 6 months cultured. In another study, Rahman *et al.* (2013) found that the net benefit BDT 1,00,784 to 4,43,458 ha^{-1} from 6 months monoculture of Thai koi (*A. testudineus*) in northern Bangladesh.

Significantly ($P<0.05$) higher BCR were also recorded in T_1 (1.68) followed by the T_2 (1.56) and lowest in T_3 (1.39), this is due to less production cost then T_2 and T_3. Noor *et al.* (2003), Usmani *et al.* (2003), Chakraborty *et al.* (2005) and Kunda *et al.* (2014) found more or less similar results of our findings.

Based on the results of growth performances, feed utilization parameters and benefit cost ratio it can be said that for shing (*H. fossilis*) polyculture 500 individuals dec^{-1} stocking density would be the best recommended stocking density for the fish farmers of northern regions.



CHAPTER 6

SUMMARY AND CONCLUSION

CHAPTER 6

SUMMERY AND CONCLUSION

A study was conducted to evaluate the growth performances of Shing in polyculture system with magur, tilapia and shorputi at three different stocking densities over a period of 150 days at Saidpur and Dimla Upazila of Nilphamari district and Taragonj Upazila of Rangpur district. The water quality parameters, growth performance and body proximate composition of shing were investigated under three different stocking densities such as 500 (T_1), 600 (T_2) and 700 (T_3) fingerlings dec^{-1} each having three replicates in the farmer's seasonal grow-out ponds. Throughout the culture period all the fish were fed with a commercial feed (Lily Fish Feed) which containing 35.53 % protein.

During the culture period, the water quality parameters such as temperature, transparency, pH, dissolved oxygen, total alkalinity, hardness and ammonia content were ranged as 27.62-28.58°C; 26.87-28.40 cm; 7.22-7.57; 5.30-5.85 (mg L^{-1}), 98.30-108.20 (mgL^{-1}), 37.80-41.30 (mgL^{-1}) and 0.15-0.18 (mg L^{-1}) respectively. Although total alkalinity, hardness and ammonia content were varied significantly, all the water quality parameters of the experimental ponds were within the acceptable ranges for fish culture.

For evaluating the growth performance of shing in the polyculture system sampling was done in every 15 days interval. The average weight gain of shing at the harvesting time was also recorded. The weight gain (62.15±0.64), SGR (2.04±0.01) were found to be highest in T_1 where the stocking density was lowest (500 fingerling dec^{-1}) compared to the higher stocking density T_2 (600 fingerling dec^{-1}) and T_3 (700 fingerling dec^{-1}). Although feeding rates, frequency and other species combination were same in all

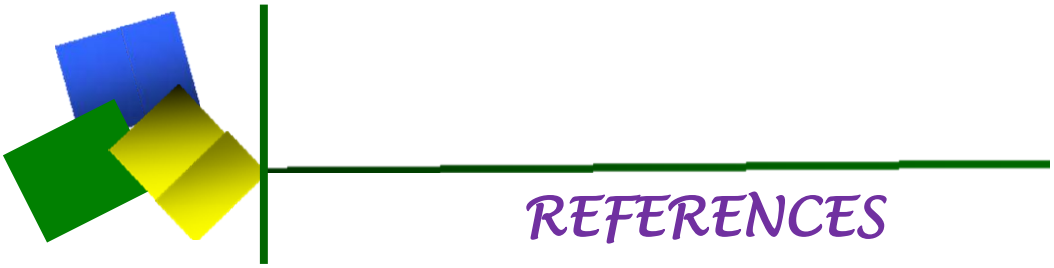
treatments, T_1 showed a significant variation in growth performances than other two treatments.

The significantly higher survival rate of shing was observed in T_1 where the stocking density was less than the other two treatments. Although production of *H. fossilis* were found higher in T_2 (6547±161.92 kg ha^{-1}) followed by T_1 (6274.69±133.41 kg ha^{-1}) and T_3 (6036.65±65.62 kg ha^{-1}) but there were no significant difference ($P>0.05$) between T_2 and T_1, while the net profit and BCR was higher in T_1 followed by T_2 and T_3.

In the present experiment, it was observed that the survival, growth and production were inversely related to the stocking densities of *H. fossilis* fingerlings in earthen ponds. From the result of the current study, it may be suggested that the stocking density 500 fingerlings dec^{-1} are sufficient for the polyculture of *H. fossilis*. The observed poor growth, survival and production at higher stocking densities might be due to competition for space, stressful condition for supplementary feed, environmental stress and unavailability of natural food. However, the optimization of stocking density for the polyculture of short cycle species *H. fossilis* will not only provide financial support but also will give quick return to the poor fish farmer in the northern region of Bangladesh. Therefore, through the application of the present findings several unused water bodies (seasonal ponds) in the rural areas of northern region of Bangladesh can be used effectively by the poor fish farmers, hence the sustainable culture of *H. fossilis* will be possible which ultimately helpful towards the protection of this species from extinction.

In order to validate the findings of this research more field trial will be needed. As a follow up to this current research the following future research is proposed:

- Effect of stocking density on the health status of shing (*H. fossilis*) under polyculture system.



REFERENCES

REFERENCES

Ahmed GU, Akter MN, Nipa SA and Hossain MM. 2009. Investigation on health condition of a freshwater eel, *Monopterus cuchia* from Ailee Beel, Mymensingh, Bangladesh. Journal of Bangladesh Agriculture University. 7(2): 419-424.

Ahmed GU, Khatun T, Hossain MB and Samsuddin M. 2012. Health condition of a farmed tilapia (*O. niloticus*) in earthen ponds. Bangladesh Journal of Fisheries. 12: 287-293.

Akand AM, Hasan MR and Habib MAB. 1991. Utilization of carbohydrate and lipid as dietary energy sources by stinging catfish (*H. fossilis*). In Fish nutrition research in Asia, Fourth Asian Fish Nutrition Workshop. Asian Fisheries Society, Manila, Philippines. pp. 93-100.

Akhteruzzaman M. 1988. A study on the production of Koi-Fish (*A. testudineus*) under semi-intensive culture system. Bangladesh Journal of Zoolology. 3: 39-43.

Alam M, Barua G, Hoque MM and Sarker AL. 1995. The effect of stocking density on the growth and survival of Magur (*C. batrachus*, Linnaeus). Bangladesh Journal of Fisheries Research. 15-18(1-2): 65-71.

Alikunhi KH. 1957. Fish culture in India Farm, Bull. No. 20. Indian Council of Agriculture Research, New Delhi. pp. 144.

Alim A. 2013. Effects of stocking density on growth and production performance of mono-sex tilapia (*O. niloticus*) in ponds. MS Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh.

AOAC. 1997. Association of official Analytical Chemist. Official Methods of Analysis of AOAC International, 16thedn, Arlington, VA, USA. 1: 1-3

Asadujjaman M, Wahab MA, Fatema MK, Hossain MB and Azam AKMS. 2013. Effects of stocking density on growth performance and production of Mola (*A. mola*). Sains Malaysiana. 42(11): 1565-1569.

Azad MAK, Rahman MR, Rahman Z, Kader MA, Haque MM and Alam MJ. 2004. Polyculture of Carp, tilapia and pangas using low cost inputs. Pakistan Journal of Biological Sciences. 7: 1918-1926.

Backiel T and Le Cren ED. 1978. Some density relationship for the population parameters. Ecology of Freshwater Fish production. Blackwell scientific publications Oxford. pp. 279-302.

BBS. 2000. Bangladesh Bureau of Statistics. http://www.bbs.gov.bd/home.aspx.

Bhuiyan BR.1970. Physico-chemical qualities of water of some ancient tanks in sibsagar, Assam. Environmental Health.12: 129-134.

Boyd CE. 1982. Water Quality Management for Pond Fish Culture. Elsevier. The Netherlands. pp. 318.

Brett JR. 1979. Environmental factors and growth in Environmental relations and behavior. Fish Physiology. 6: 599-677.

Burgess WE. 1989. An atlas of freshwater and marine catfishes-A preliminary survey of the Siluriformes. TFH Publications, Neptune City, New Jersey.

Chakraborty BK and Nur NN. 2012. Growth and yield performance of shing (*H. fossilis*) and Koi (*A. testudineus*) in Bangladesh under semi-intensive culture system. International Journal of Agriculture, Research Innovation and Technology. 2(2): 15-24.

Chakraborty BK, Miah MI, Mirza MJA and Habib MAB. 2005. Growth, yield and returns to *P. sarana* (Hamilton) Sarpunti, in Bangladesh under semi intensive aquaculture. Asian Fisheries Science. 18: 307-322.

Chakraborty BK. 2010. Status and position of aquatic biodiversity of four beels and its floodplain level of northern Bangladesh with a good practice of beel nurseries. International Advances in Environmental Research. Nova Science Publishers, New York, USA. 8: 121-164.

Chareontesprasit N, Jiwyam W, Khongsai S and Tiebsri S. 1997. Optimum dietary protein level and stocking density of climbing perch (*A. testudineus*) in cage culture. Khon Kaen Agriculture Journal. 25(1): 42-47.

Chattopadhya DN, Mohapatra BC, Adhikari S, Panij KC, Jena K and Eknath AE. 2013. Effects of stocking density of *L. rohita* on survival, growth and production in cages. Aquaculture. 21(1): 19-29.

Das P, Islam MA and Maugham GU. 1992. Induced breeding and fry rearing of catfish (*C. batrachus*, Linn.). Bangladesh Journal of Fisheries Research. 20(1): 87-95.

Dev M. 2009. Polyculture of carps with tilapia, MS Thesis, Department of Aquaculture.

DoF. 1996. Matsha pakkha Sankalan (Bengali). Department of Fisheries, Ministry of Fisheries and Livestock, Government of the people's Republic of Bangladesh. 11: 34-81.

DoF. 2015. National fish week, compendium (In Bengali). Dhaka: Department of Fisheries, Ministry of Fisheries and Livestock, Government of Bangladesh.

FAO. 2014. Fisheries and Aquaculture topics, composition of fish, topics fact sheet, Rome. Italy: FAO. http://www.fao.org/fishery/topic/14869/en.

Folch J, Lees M and Solane Stanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry. 226(1): 497-509.

FRSS. 2016. Fisheries resources survey system. Fisheries statistical report of Bangladesh, Department of Fisheries. 32: 1-57.

Haque M. 2014. Optimization of stocking density on growth and production of mono-sex tilapia in pond, MS Thesis, Department of Aquaculture, BAU, Mymensingh. pp. 51.

Haque MM and Barua G. 1989. Rearing of shing (*H. fossilis*, Bloch) fry under laboratory condition-II. Bangladesh Journal of Fisheries Research. 12: 67-72.

Haque MM and Razzaque MA. 1998. Auger manual for farming system development, Bangladesh Agricultural Research Council, Dhaka-1215, Bangladesh. pp. 40.

Haque MR, Rahman MS, Islam MS, Ali MM, Islam MR and Shahabuddin AM. 2015. Production and growth performances of carps in different stocking densities of polyculture. World Journal of Zoology. 10(4): 247-251.

Haque MZ, Rahman MA, Hossain MM and Rahman MA. 1994. Effect of stocking densities on the growth and survival of mirror carp (*C. carpio* var. *specularis)* in rearing ponds. Bangladesh Journal of Zoology. 22: 109-116.

Hossain MA, Alam MR, Haque MR and Mehbub MF. 2013. Study on present status of carp-SIS polyculture in Dinajpur district of Bangladesh. Bangladesh Journal of Research Publication. 9(2): 123-130.

Hossain MAR. 2014. An overview of fisheries sector of Bangladesh. Research in Agriculture, Livestock and Fisheries. 1(1): 109-126.

Hossain MI, Ara J, Kamal BMM, Tumpa AS and Hossain MY. 2014. Effects of fry stocking densities on growth, survival rate and production of *H. molitrix*, *C. carpio* var. *specularis* and *L. rohita* in earthen ponds at Natore fish farm, Natore. Bangladesh International Journal of Fisheries and Aquatic Studies. 2(1): 106-112.

Hossain MM. 2009. Effects of partial replacement of fishmeal by fermented soybean meal and squid by-product blend in the diet of climbing perch (*A. testudineus*). MS Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh, Bangladesh. pp. 48.

Houde ED. 1975. Effect of stocking density and food on survival, growth and yield of laboratory reared of sea Bream, *A. homboidales* (L.). Journal of Zoology. 7: 115-127.

Hussain MG and Mazid MA. 2001. Genetics improvement and conservation of carp species in Bangladesh. Bangladesh Fisheries Research Institute, Mymensingh. pp. 56.

INFS. 1997. Food value of national food stuff (Institute of Nutrition and Food Science University of Dhaka). pp. 33.

IUCN. 2000. Red book of threatened fish of Bangladesh. The world conservation Union. pp. 61.

Jahan S. 2012. Growth performance of climbing perch (*A. testudineus*) in monoculture and polyculture system, MS Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh.

Jannat MK, Rahman MM, Bashar MA, Hasan MN, Ahmed F and Hossain MY. 2012. Effects of stocking density on survival, growth and production of Thai climbing perch (*A. testudineus*) under fed ponds. Sains Malaysiana. 41(10): 1205-1210.

Jannat MK. 2009. Effects of stocking density on the growth, survival and production of Thai koi (*A. testudineus*) fed on formulated diet in farmer's ponds, MS Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh.

Jena JK, Aravindakshan PK, Chandra S, Muduli HK and Ayyappam S. 1998. Comparative evaluation of growth and survival of Indian major carps and exotic carps in raising fingerlings. Journal of Aquaculture Tropics. 13: 143-150.

Jena JK, Ayyappam S, Aravindkshan PK and Muduli HK. 2001. Comparative evaluation of growth, survival and production of carp species at different stocking densities under polyculture. Indian Journal of Fisheries. 48(1): 17-25.

Jhingran VG. 1975. Aquaculture lecture, summer intensive fresh water fish culture, Cuttack organized by Central. Inland Fisheries Research Institute, Barracckpore (ICCR), West Bengal, India. pp. 12.

Kawamoto NY, Incuye Y and Nakanishi S. 1957. Studies on effects by the ponds areas and densities of fish in the water upon the growth rate of carp (*C. carpio*), Handbook of freshwater, Fisheries Biology. Lowasae University press. 1: 752-759.

Khan MN, Islam AKMS and Hossain MG. 2003. Marginal analysis of culture of stinging catfish (*H. fossilis,* Bloch): Effect of different stocking densities in earthen ponds. Pakistan Journal of Biological Science. 6(7): 666-670.

Kohli MPS and Goswami UC. 1989. Studies on age and growth of an air-breathing catfish (*H. fossilis)*. Journal of Inland Fisheries Society, India. 21: 17-24.

Kohinoor AHM, Akhteruzaman M and Saha MS. 1993. Production of Puntius gonionotus (Bleeker) in ponds. Bangldesh Journal of Zoology. 21(2):77-83.

Kohinoor AHM, Islam ML, Wahab MA and Thilsted SH. 1998. Effect of Mola (*A. mola,* Ham.) on the growth and production of carp in polyculture. Bangladesh Journal of Fisheries Research. 2: 119-126.

Kohinoor AHM, Khan MM, Yeasmine S, Mondol P and Islam MS. 2012. Effects of stocking density on growth and production performance of indigenous stinging catfish (*H. fossilis)*. International Journal of Agriculture Research Innovation and Technology. 2(2): 9-14.

Kohinoor AHM, Modak PC and Hussain MG. 1998. Growth and production performance of red tilapia and Nile tilapia (*O. niloticus*) under low input culture system. Bangladesh Journal of Fisheries Research. 3(1): 11-17.

Kohinoor AHM, Rahman MM, Islam MS and Mahmud Y. 2016. Growth and production performance of climbing perch Thai Koi and Vietnamese Koi Strain (*A. testudineus*) in Bangladesh. International Journal of Fisheries and Aquatic Studies. 4(1): 354-357.

Kohinoor AHM, Wahab MA, Islam, AL and Thilsted SH. 2001. Culture potential of mola (*A. mola*), Chela, (*C. cachinus*) and Punti (*P. sophore*) under mono culture system. Bangladesh Journal of Fisheries. 5(2): 123-134.

Kohinoor AHM. 2000. Development of culture technology of three small indigenous fish mola (*A. mola*), punti (*P. sophore*) and chela (*C. cachius*) with nites on some aspect of their biology, PhD Thesis, Department of Fisheries Management, Mymensingh. pp. 287.

Kunda M, Shah MNA and Mazumder SK. 2014. Production potential of nutrient rich small indigenous species Dhela (*O. cotio*) in carp polyculture system. Journal of Fish and Marine Science. 6(5): 453-460.

Lakshmanam MAV, Sukumaran KK, Murty DS, Chakroborty DP and Philipose MT. 1971. Preliminary observations on intensive fish farming in freshwater ponds by the composite culture of Indian and exotic species. Journal of Inland Fisheries Society of India. 3: 1–21.

Le Cren ED. 1965. Some factors regulating the size of population of Freshwater Mitteilungen. International Vereinigung fur Theoretische and Angewandte Limnology. pp. 1-29.

Maghna MSA. 2012. Studies on the stocking density of hormone treated tilapia (*O. niloticus*) fry, MS Thesis, Department of Aquaculture, Bangladesh Agriculture University, Mymensingh. pp. 57.

Michael GR. 1969. Seasonal treands in physico-chemical factors and plankton of freshwater fish ponds and their role in fish culture. Hydro-biological Journal. 33(1): 144-160.

Mollah MFA and Haque AKM. 1978. Studies on monthly variations of plankton in relation to the Physico-chemical conditions of water and bottom soil of two ponds. Bangladesh Journal of Fisheries Research. 1(1): 29-39.

Mollah MFA. 1985. Effects of stocking density and water depth on the growth and survival of fresh water catfish (*C. macrocephalus*, Guther) larvae. Indian Journal of Fisheries. 32(1): 1-17.

Monir MS and Rahman S. 2015. Effect of stocking density on growth, survival and production of shing (*H. fossilis*) fingerlings under nursery ponds in Northern region of Bangladesh. International Journal of Fisheries and Aquatic Studies. 2(3): 81-86.

Mookerjee HK and Mazumdar SR. 1950. Some aspect of the life history of *C. batrachus*. Proceeding of the Zoological Society of Bengal. 3: 71-84.

Narejo NT, Salma MA, Sabur MA, Rahmatullah SM. 2005. Effect of stocking density on growth and survival of indigenous catfish (*H. fossilis*) reared in cemented cistern fed on formulated feed. Pakistan Journal of Zoology. 37(1): 49-52.

Noor Khan M, Saiful Islam AKM and Hussain MG. 2003. Marginal analysis of culture of stinging catfish (*Heteropneustes fossilis*, Bloch) effect of different stocking densities in earthen ponds. Pakistan Journal of Biological Science. 6: 666-670.

Pathak SC. 1978. Culture of *A. testudineus* in cemented cistern. In 4th workshop of all India Coordinated Research project on air breathing fish culture at CIFRI, Barrack pore, India. pp. 1-10.

Patra BC. 1993. Satiation time, appetite and daily pattern of feed intake and faces release by an air breathing fish (*A. testudineus*). Journal of Aquaculture. 8(1): 41-46.

Paul S. 1998. Comparison between carp polyculture system with silver carp (*H. molitrix*) and small indigenous fish mola (*A. mola*), M.S Thesis, Department of Fisheries Management. Bangladesh Agricultural University, Mymensingh. pp. 71.

Powell MR. 1972. Cage and race-way culture of striped bass in brackish water in Alabama Pro. 26th Annual Conferences Southeast Association Game Fish Committee. pp. 533-565.

Rahim A. 2010. Effects of stocking density on growth and production performance of mono-sex tilapia (*O. niloticus*) in ponds, MS Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. pp. 61.

Rahman MA, Gheyasuddin H, Rashid MH and Chowdhury MFZ. 1982. Proximate composition and native quality of freshwater Zeol fishes of Bangladesh. Bangladesh Journal of Fisheries. 25: 37-43.

Rahman MM. 2000. Effect of species combination on pond ecology and growth of fish in carp-SIS polyculture system, MS Thesis, Department of Fisheries Management, Bangladesh Agriculture University, Mymensingh. pp. 56.

Rahman MM, Mondal DK, Amin MR and Billah M. 2016. Effects of stocking density on growth and production of shing (*H. fossilis*) in ponds. Asian Australas Journal of Bioscience Biotechnology. 1(2): 380-385.

Rahman MS. 1992. Water Quality Management in Aquaculture. Published by Bangladesh Rural Advancement Committee. pp. 75.

Rahman S and Monir MS. 2013. Effect of stocking density on survival, growth and production of Thai koi (*A. testudineus*) fingerlings under nursery ponds management in northern regions of Bangladesh. Journal of Experimental Biology and Agricultural Sciences. 1(6): 466-472.

Rahman S, Monir MS and Khan MH. 2013. Culture potentials of climbing perch, Thai koi (*A. testudineus)* under different stocking densities in northern regions of Bangladesh. Journal of Experimental Biology and Agricultural Science. 1(3): 203-208.

Rahman S, Monir MS and Mou MH. 2014. Culture potentials of stinging catfish Shing (*H. fossilis*) under different stocking densities in Northern Region of Bangladesh. 3(2): 2319–4758.

Rouse DB, Nagar GOE and Mulla MA. 1987. Effects of stocking size and density of tilapia on *M. rosenbergii* in polyculture. Journal of the World Aquaculture Society. 18(2): 57–60.

Roy NC, Kohinoor AHM, Wahab MA and Thilsted SH. 2002. Evaluation of performance of Carp-SIS Polyculture technology in the rural farmer's pond. Asian Fisheries Science. 15: 41-50.

Roy NC, Wahab MA, Khatun H and Thislsted SH. 2003. Economics of carp SIS polyculture in rural farmer s pond. Pakistan Journal of Biological Sciences. pp. 2333-9721.

Saha KC and Guha BC. 1939. Nutritional investigation on Bengal fish. Indian Journal of Medical Research. 26: 921-927.

Shoko AP, Limbu SM, Mrosso HDJ, Mkenda AF and Mgaya YD. 2014. Effect of stocking density on growth, production and economic benefits of mixed sex nile tilapia (*O. niloticus*) and African catfish (*C. gariepinus*) in polyculture and monoculture. Aquaculture Research. pp. 1-15.

Siddik MAB and Khan MMR. 2007. Over-wintering performance of mixed sex and monosex tilapia (*O. niloticus*) in Bangladesh. Bangladesh Journal of Fisheries. 11(2): 153-162.

Siddiquee MMR, Rahman MF, Jahan N, Jalal KCA, Amin SMN and Arshad A. 2012. Impact of Exotic Carps in the Polyculture with Indigenous Carps: Competition for Food. Pakistan Journal of Biological Sciences. 15: 568-575.

Tarnchalanukit W, Chuapoehuk W and Suraniranat P. 1983. Pal duck Dan culture in circular concrete ponds with water recalculating system, PhD Thesis, Department of Agriculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand.

Tharakan B and Joy KP. 1996. Effects of mammalian gonadotropin-releasing hormone analogue, pimozide and the combination on plasma gonadotropin levels in different seasons and induction of ovulation in female catfish. Journal of Fisheries Biology. 48: 623-632.

Tripathi SD, Dutta A, Sen Gupta KK and Pattra S. 1979. High density rearing of rohu spawns in village ponds. In: Symposium of Inland Aquaculture (Abstracts).CIFRI Barrack pore, India. pp. 12-13.

Usmani N, Jafrim AK and Khan MA. 2003. Nutrient digestibility studies in *H. fossilis* (Bloch), *C. batrachus* (Linnaeus) and *C. gariepinus* (Burchell). Aquaculture. 34: 1365-2109.

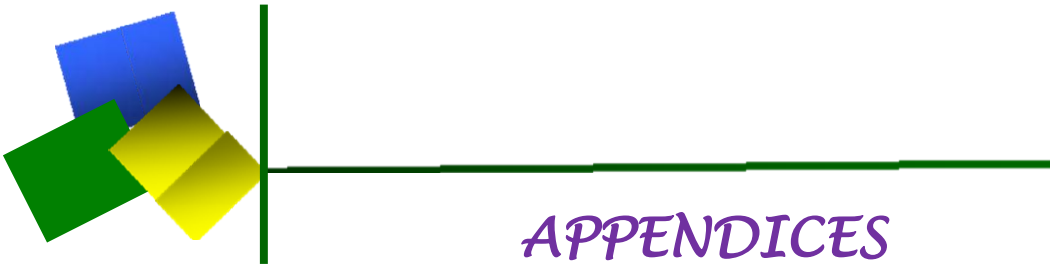
Vijayakumar C, Sridhar S and Haniffa MA. 1998. Low cost breeding and hatching techniques of the catfish (*H. fossilis*) for small-scale farmers, Naga. 21: 15-17.

Wahab MA, Ahmed ZF, Haque MS, Haque MA and Biswas BK. 1995. Effect of frequency of fertilization on the pond ecology and growth of fishes. BAU Research Progress. 9: 410-419.

Wahab MA, Thislted SH and Haque EN. 2003. Small indigenous species of fish in Bangladesh. Proceedings of BAU-ENRECA/DANIDA workshop, 30-31 October 2003, Bangladesh Agricultural University, Mymensingh, Bangladesh. pp. 150.

Wahid MI, Haq MS, Wahab MA and Ahmed ZF.1997. Effects of fertilizer treatments on the water quality and fish production in semi-intensively managed ponds. Progress in Agriculture. 8(1-2): 61-65.

Zahidah Y, Andriani and Zidni I. 2015. Effect of density ratio on performance of nile tilapia and catfish in polyculture fish farming system. Lucrări Ştiinţifice - Universitatea de Ştiinţe Agricoleşi Medicină Veterinară, Seria Zootehnie. 63: 246-249.



APPENDICES

APPENDICES

Appendix A: Water Quality Analysis

A.1 Analysis of alkalinity

Determination of Phenolphthalein Alkalinity

- Remove the cap from the small plastic vessel. Rinse the plastic vessel with water sample, fill to the 5 ml mark and replace the cap.
- Add 1 drop of phenolphthalein indicator through the cap port and mix carefully swirling the vessel in tight circles, record the phenolphthalein alkalinity as zero and proceed with the procedure for the determination of Total Alkalinity. If the solution is pink or red, proceed to next step.
- Take the titration series and push plunger completely into HI 3811-0 solution and plunger seal is on the 0 ml mark of the syringe.
- Place syringe tip into the cap port of the plastic vessel and slowly add the titration solution drop wise, swirling to mix after each drop. Continue adding titration solution in the plastic vessel turns colorless.
- Read off the millimeters of titration solution from the syringe scale and multiply by 300 to obtain mg L^{-1} (ppm) $CaCO_3$.

Determination of Total Alkalinity

- Remove the cap from the plastic vessel. Rinse the plastic vessel with water sample, fill to the 5 ml mark and replace the cap.

- Through the cap port, add 1 drop of bromophenol blue indicator and mix. If the solution is yellow, then it is acidic and an acidity test must be carried out (see HI 3820-Hanna Acidity Test Kit). If the solution is green or blue, then precede next step.

- Take the titration syringe and push the plunger completely into the syringe. Insert the tip into HI 3811-0 and pull the plunger out until the 0 ml mark of the syringe.

- Place the syringe tip into the cap port of the plastic vessel and slowly add the titration solution drop wise, swirling to mix after each drop. Continue adding titration solution until the solution in the plastic vessel turns yellow.

- Read the millimeters of titration solution from the syringe scale and multiply by 300 to obtain mg L^{-1} (ppm) $CaCO_3$.

A.2 Analysis of hardness

High range 0 to 300 mg L^{-1} $caco_3$

- Remove the cap from the small plastic beaker. Rinse the plastic beaker with the water sample, fill to the 5 ml mark and replace the cap.

- Add 5 drops of Hardness Buffer through the cap port and mix carefully swirling the beaker in tight circles.

- Add 1 drop of calmagite indicator through the cap port and mix as described above. The solution becomes a red-violet color.

- Take the titration syringe and push the plunger completely into the syringe. Insert tip into HI 3812-0 EDTA solution and pull the plunger out until the lower edge of the seal is on the 0 ml mark of the syringe.

- Place the syringe tip into the cap port of the plastic beaker and slowly add the titration solution drop wise, swirling to mix after each drop.

- Continue adding the titration solution until the solution becomes purple, then mix for 15 seconds after each additional drop until the solution turns blue.

- Read off the mili-liters of titration solution from the syringe scale and multiply by 300 to obtain mg L^{-1} (ppm) $caco_3$.

  ….X300= $caco_3$

A.3 Analysis of Ammonia

- Removed the cap from the plastic beaker. Rinsed the plastic beaker with water sample before filling it up to the 10 ml mark.
- Added 2 drops of Ammonia Reagent 1 for fresh water replace the cap and mix by carefully swirling the beaker in tight circles.
- Added 8 drops of Nessler Reagent replace the cap and mix by carefully swirling the beaker.
- Removed the cap and transfer the solution into the color comparator cube. Wait for 5 minutes to allow color to develop.
- Determine which color matches, the solution in the cube and record the results in mgL^{-1} (ppm).
- It is better to match the color with a white sheet of about 10 cm behind the comparator.

Appendix B: Proximate Composition Analysis (AOAC)

B.1 Determination of Moisture content

- At first marking the empty petridish according the sample used, weighed out these petridish by using an electronic balance and was recorded.
- Then the sample ingredients were weighed out in the clean weighed petridish by using the balance.
- Then the samples were placed in a hot air oven at 105°C for 12 hours.
- After 12 hours the samples were carefully taken out from the oven by using a specialized forceps and were kept in a desiccator for cooling.
- Finally the weight of each sample with petridish was taken again.
- The differences in weight were represents the moisture content of the samples.
- The percent moisture content of whole body fish sample was then calculated by using the following formula:

% of moisture = E/C x 100

Where,

E= Weight of moisture.

C= Weight of sample.

B.2 Determination of Crude Protein (Kjeldhal method)

- The sample as much as 0.1 g is weighted on ash free paper and recorded.
- Each sample is made in triplicate.
- The sample is then taken in Kjeldahl flasks and numbered.
- One teaspoon of catalyst containing K_2SO_4 + Cu SO_4 and 25 ml sulfuric acid is added to each Kjeldahl flask.
- Then, Kjeldahl flasks are placed on Kjeldahl Digestion unit to digest the sample.
- The flasks are heated for 20 minutes at 250°C and shaken properly.
- The temperature is increased up to 350-380°C for 1-2 hour, or until the color of the solution become light green.
- The samples, then, are digested for another 45 minutes.
- The heater is switched off and left for 10-20 minutes until all the flasks cool down to room temperature.
- The flasks are then connected to the Kjeldahl Distillation unit. 300 ml distilled water is added to each flask automatically in the distillation unit.
- For the distillation process, 100 ml of 40% NaOH is added automatically from the jar contain 40% NaOH in each Kjeldahl flask.
- 25 ml of boric acid and 2-3 drops of indicator (methylene red + bromocresol blue) are prepared in Erlenmeyer flasks, connected to the end duct of the apparatus.

- Make sure the duct is immersed into the solution during the distillation.
- The distillation process is conducted until as much as 75 ml boric acid solution is collected.
- All the distillates are titrated with 0.1 N HCL until the color of the solution turn grayish blue.
- Record the volume of HCL used for titration. Repeat the analysis triplicate for each sample.
- Use the following formula for the calculation of crude protein content

$$\%\text{Nitrogen} = \frac{\text{ml. of titrant used} \times \text{normality of titrant X milli equivalent weightt of Nitrogen}}{\text{Weight of the sample (g)}} \times 100$$

% Crude protein in sample = % Nitrogen X 6.25

B.3 Determination of Crude Lipid

- About 1 g of each sample is weighted in triplicate. The sample is homogenized with 60 ml of chloroform: methanol solution at the ratio of 2:1 for 2 minutes.
- Homogenate sample is filtrated through Buchner flask using filter paper (whatman qualitative No.1).
- Fat free residue left on the filter paper. The sample is washed with 40 ml solvent (chloroform: methanol) and transferred to a separating funnel. 20 ml distilled water is added and the mixture is shaken properly for 1 minute.
- The funnel is vertically left for separation of two phases.
- The upper phase is a mixture of distilled water and methanol, the lower phase is extracted lipid and chloroform.

- After 2-3 hours, the lower phase is collected in a beaker which has been weighed. The beaker together with the sample inside is placed in the oven at 80 °C to evaporate the chloroform.

- After 4 hours, the beaker with the dried lipid is taken out and kept in the desiccator until cool. Record the beaker weight. Crude lipid is calculated as:

$$\%\ \text{Crude lipid} = \frac{\text{Weight of beaker with lipid (g)} - \text{Weight of empty beaker (g)}}{\text{Weight of sample}} \times 100$$

B.4 Determination of Ash Content

- The porcelain crucible is dried in the oven at 100°C for 1 hour.

- After cool it in a desiccator, the weight of each crucible is recorded. About 1 g of sample is weighed and put in the crucible.

- The crucible together with sample is heated up in the muffle furnace at 550°C for 5 hours. After 5 hours, the furnace is switched off.

- After cool down the furnace the crucible is taken out and left in the desiccator until cool.

- The weight of porcelain crucible with the remaining ash is recorded.

- % ash is calculate

$$\%\ \text{Ash} = \frac{\text{Weight of crucible with ash(g)} - \text{Weight of empty crucible (g)}}{\text{Weight of sample (g)}} \times 100$$