EFFECT OF STOCKING DENSITY ON GROWTH, SURVIVAL AND PRODUCTION OF THAI KOI (*Anabas testudineus*) FRY IN THE NURSERY PONDS OF NILPHAMARI DISTRICT OF BANGLADESH

A Thesis

By

NAJIDA KHATUN
Examination Roll No. 1605211
Session: 2016-2017
Semester: January- June, 2017

MASTER OF SCIENCE (MS)

IN

AQUACULTURE

DEPARTMENT OF AQUACULTURE

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY
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June 2017

DEDICATED

TO

MY BELOVED PARENTS

DECLARATION

I declare that this MS thesis entitled Effect of stocking density on growth, survival and production of Thai koi (*Anabas testudineus*) fry in the nursery ponds of Nilphamari district 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

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The Author

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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
FCR	Food Conversion Ratio
FRSS	Fisheries Research Survey System
g	Gram
GIFT	Genetically Improved Farmed Tilapia
Ha	Hectare
Kg	Kilogram
L	Liter
mg	Milligram
MOFA	Ministry of Food and Agriculture
MT	Metric Tons
NFP	National Fisheries Policy
PER	Protein Efficiency Ratio
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
USA	United States of America

ABSTRACT

The present study was carried out to assess the effect of stocking density on the growth, survival and production of Thai koi (*Anabas testudineus*) in the farmer's seasonal nursery ponds of Nilphamari district for a period of 30 days. There were three treatments each having three replications. Around 0.2g weight of Thai koi were stocked at the rate of 1200, 1400 and 1600 fry per decimal in T_1, T_2 and T_3 respectively. All the experimental fish were fed with a commercial feed containing 35.53% crude protein (nursery Lily fish feed) three times daily at a rate of 25% body weight for the first 10 days, 15% for the second 10 days, and finally 10% for the last 10 days. During the experimental period the water quality parameters were suitable for fish culture. The water temperatures were ranged from 27.20 to 27.60 ℃; transparency 26.98 to 33.50 cm, pH 6.34 to 8.70, dissolved oxygen 5.02 to 5.17 mgL^{-1}; and total alkalinity 99.40 to 109.10 mgL^{-1}. Significantly higher ($P<0.05$) transparency, NH_3, alkalinity and hardness were observed in the higher stocking density compared to those treatments where the stocking density were lower. The weight gain (6.35±0.90g), specific growth rate (SGR 11.26±0.41 % per day) and protein efficiency ratio (PER 4.59±0.71) were significantly increased ($P<0.05$) after 30 days feeding trial in the treatment T_1 compared with the other treatment groups. While, a significantly lowest ($P<0.05$) feed conversion ratio (FCR) was observed in the treatment T_1 followed by T_2 and T_3. Significantly the highest ($P<0.05$) survival and production were observed in the treatment T_1 compared with the remaining two treatments. At the end of the experiment the benefit-cost ratio (BCR) revealed that best BCR was 2.04 in T_1 which did not differed significantly ($P>0.05$) when compared with T_2. Therefore, the results of this study indicated that the lowest stocking density (1200 fry dec^{-1}) seems to have a more positive influence on the enhancement of the growth performance and feed utilization of Thai koi (*A. testudineus*) fry in the seasonal farmer's nursery pond.

KEY WORDS: Stocking density, growth, survival, *Anabas testudineus,* nursery ponds.

CHAPTER 1

INTRODUCTION

Aquaculture remains one of the fastest-growing food producing sectors in the global fish production and continues to improve faster than world population growth (FAO, 2014). Aquaculture has been responsible for the significant growth in the supply of fish for human consumption. Whereas aquaculture provided only 7% of fish for human consumption in 1974, this share had increased to 26% in 1994 and 39% in 2004, and by 2030 its amount will be increased to 60%. The Production of aquatic animals from aquaculture in 2014 amounted to 73.8 million tones, with estimated first-sale values of US$ 160.2 billion. China accounted for 45.5 million tonnes in 2014 or more than 60% of global fish production from aquaculture. Other major producers were India, Vietnam, Bangladesh and Egypt.

Aquaculture is the farming of fish and other aquatic organisms, with 'farming' implying some form of intervention to increase productions, and some form of private rights of the stock under intervention (Beveridge and Little, 2002). The entire area of inland closed (culture) water fisheries bodies in Bangladesh is 0.29 million ha with littoral shrimp farms (National Fisheries Policy, 1998). In Bangladesh, the overall pond area is 3,72,397 ha and ox-bow lakes (baors) are 5,488 ha (DoF, 2016). Aquaculture now provides around half of the fish for direct human consumption and is set to grow further. Over the last 10 years, the average growth rate of fisheries is 5.4%, while aquaculture has grown 8.2% (FRSS, 2015). In recent years, the bulk of the production has been obtained from marine (16.78%) and freshwater (83.22%) wild capture fisheries. Bangladesh was the 5th in world aquaculture production (closed) which accounted more than half of the country's total fish production (55.15%) (DoF, 2016). The inland pond culture is the mainstay of aquaculture in Bangladesh, contributing more than 80% of the total aquaculture production, which is currently dominated by exotic and indigenous carps, Mekong pangas and tilapia. Pond aquaculture is mostly practiced in closed water fisheries in Bangladesh and contributed 43.79% (1,613,240 MT) to the total fish production in 2014-15 (DoF, 2016)

In the world the most rapidly growing sectors are aquaculture. Fish farmers are trying to grow more production of fish yields to earn more profit by introducing different kinds of exotic fishes. Exotic fish are alien species which is not native and belonging by nature or origin to another part of the world are brought from abroad or foreign or strange. The purpose of introducing fishes is to fill up the vacant niches of the water body, to obtain more production and to control of weeds insects and pests. In Bangladesh aquaculture there are about 92 species of exotic fishes are cultured (Bijukumar, 2000) and contributes about 17% to global food aquaculture production (Shelton and Rothbard, 2006).

With this scenario, small exotic fish species of Climbing perch (*A. testiduneus*) was imported from Thailand into Bangladesh in 2002 and it locally bears the name of Thai koi. It contains high amount of physiologically available iron and copper essentially needed for hemoglobin synthesis, besides, it possesses easily digestible fat of very low melting point and many essential amino acid**s** (Saha, 1971). Thai koi looks like our indigenous koi but its body covers with a gray color and small black spots. This fish can be cultured in ponds, tanks and cages successfully. It is tough to culture our indigenous koi in pond providing supplementary feed rather than possible to culture, Thai koi in pond successfully supplying artificial feed. The climbing perch (*A. testudineus*) or koi is considered as an important economic fish species which has high market value and good nutritional profile. The reasons behind the greater expansion of Thai koi culture in

Bangladesh are; a) The fish can tolerate adverse environmental conditions easily; b) It can be cultured at high stocking density; c) It is cultivable under deep or shallow water; d) It can also survived in low oxygen level; e) It can be marketed at live condition; f) It is short cycle species and can be harvested within 3-4 months; g) It is suitable for culturing in seasonal ponds.

About 55% ponds of the Nilphamari districts of Bangladesh are seasonal of which 60% retained water for 4-6 months, while 40% retained for 6 to 9 months in a year and even more in some areas (BBS, 2000) make this area very potential for culturing this species. The culture of short-cycle species should be introduced to ensure the proper utilization of seasonal ponds which enhanced to get higher production. The demands for this fish are owing to their taste and nutritive values, and can be used as patient food.

Nursing stage is one of the most critical stages to produce the quality fingerling. However, it can be bred successfully in our country but the most of the farmers in our country have inadequate knowledge of the nursing of this species. Therefore, it is important to give the appropriate knowledge of nursing fry to the farmers; they will be able to produce quality fish production.

Another most important factor that has direct effects on the growth, survival and production of fish is to determine the optimum stocking density for each species (Bacheil and Le Cren, 1978). It is an established fact that the growth rate progressively increase as the stocking density decreases and vice-versa. The growth of fishes is directly dependent on the population density (Le Cren, 196 and Backeli and Le Cren, 1978), Generally direct relationship exists between food abundance and growth rate as well as between population density of the species and its growth rate tend to be inversely related (Le Cren, 1965). Stocking densities and management measures practiced by nursery pond operators in Bangladesh are not based on scientific knowledge, thus resulting in poor growth and survival of the fry. To obtain maximum economic returns it would be necessary to stock the nursery ponds at optimum stocking densities for optimum growth.

The major constraint for the sustainable development of aquaculture sector is the availability of quality fry and fingerling. As the growth and survival of fish fry and fingerlings are depend on many factors such as the maintaining of proper stocking density, application of inorganic and organic fertilizers for increasing the primary productivity of the pond and providing an adequate amount of the quality supplementary feed. No work has been undertaken to evaluate the effects of stocking density on the survival and growth of Thai koi *A. testudineus* fry in the farmer's nursery ponds of the Nilphamari district of Bangladesh. Considering the above fact, the present work was undertaken to develop a suitable and economically viable technology for the production of Thai koi *A. testudineus* fingerlings in the farmer's nursery pond of the Nilphamari district of Bangladesh. Therefore, the present study has been undertaken with the following objectives-

- To determine the effect of the stocking density on the growth performance, feed utilization and proximate composition of Thai koi in the farmer's seasonal nursery ponds.
- To evaluate the effect of the stocking density on the water quality parameters and survival rate of Thai koi in the farmers seasonal nursery ponds.
- To analyze the Benefit Cost Ratio (BCR) of nursing technologies.

CHAPTER 2

LITERATURE REVIEW

At present Thai koi has gained much popularity in Bangladesh due to its higher production and acceptable taste. The effects of different stocking densities on the growth and survival of Thai koi regarding this information are very much useful to fish farmers. Since it is comparatively a newly brought exotic species not much work have been done regarding various aspects of its nursing period in the nursery pond. The following information was reviewed in favour of the present study which has done around the world and relevant to the study.

2.1 Stocking density

Nursing practice of *A. testudineus* (Thai koi) successfully in nursing ponds with different stocking densities, the following literatures were reviewed.

Kohinoor *et al.* (2016) reported an experiment to evaluate the growth and production performance of climbing perch Thai Koi and Vietnamese Koi Strain (*A. testudineus*) in Bangladesh. The authors were tested two treatments each with three replication, having three ponds under treatment-1 (T_1) were stocked with fry of Vietnamese strain of climbing perch, while other three ponds which designated as treatment-2 (T_2) were stocked with a Thai strain of climbing perch and found that the higher growth performance and production were noted in the case of Vietnamese strain compared to the Thai strain of climbing perch.

Habib *et al.* (2015) carried out an experiment to assess the effects of stocking density on growth and production performance of cage reared Climbing Perch (*A. testudineus*) of high yielding Vietnamese Stock. The authors reported that three different stocking density such as 100 fish m^{-3}, 150 fish m^{-3} and 200 fish m^{-3} and observed the cage with 150 fishes m^{-3} showed promising growth, yield and survival rate which could be recommended to adopt.

Monir and Rahman (2015) stated the effect of stocking density on growth, survival and production of shing (*Heteropneustes fossilis*) fingerlings under nursery ponds in Northern region of Bangladesh. The authors reported thee different stocking densities were 2 million fry ha^{-1}, 2.5 million fry ha^{-1} and 3 million fry ha^{-1} in T_1, T_2 and T_3 respectively and observed 2 million fry ha^{-1} (T_1) is the most suitable stocking density for nursing of *H. fossilis* for better production as well as higher net benefits.

Daudpota *et al.* (2014) describes the effect of stocking densities on growth, production and survival rate of red tilapia in hapa at fish hatchery Chilya Thatta, Sindh, Pakistan. The authors conducted three treatment as T_1 (200 fry $hapa^{-1}$), T_2 (250 fry $hapa^{-1}$) and T_3 (300 fry $hapa^{-1}$) and found the best growth performance in T_1 which was the lowest stocking density.

Jiwyam and Nithikulworawong (2014) conducted an experiment on the stocking density dependent growth and survival of Asian red-tailed catfish (*Hemibagrus wyckioides*) fries: early nursing in cages. The authors tested different stocking densities were 25, 50, 100, 200 and 400 fish m^{-3} and found Asian river catfish performed poorly at the lowest stocking density.

Ferdous *et al.* (2014) conducted an experiment the influence of stocking density on growth indices and survival of *Oreochromis niloticus* fry for a period of 28 days. The authors were showed three different stocking densities such as 1000 fry $hapa^{-1}$ (T_1), 1500 fry $hapa^{-1}$ (T_2) and 2000 fry $hapa^{-1}$ (T_3).These experiments evaluated that stocking density had a significant effect on growth and survival rates of monosex tilapia. Fry held at the highest density exhibited lowest growth and survival rates.

Ronald *et al.* (2014) carried out an experiment on the effects of stocking density on the growth and survival of Nile tilapia (*O. niloticus*) fry at son fish farm, Uganda. In this study, the authors were tested Nile tilapia fry were randomly stocked at densities 1000, 1330, 2000, 2670, 4000 and 5330 fry m^{-3} for an experimental period for 23 days and found that increasing the stocking density of Nile tilapia fry beyond 2670 fry m^{-3} significantly affects survival and growth of fry.

Asadujjaman *et al.* (2013) evaluated an experiment to determine the effects of stocking density on growth performance and production of *Amblypharyngodon mola.* The authors were conducted three treatments designed as T_1 (145000ha^{-1}), T_2 (73000ha^{-1}) and T_3 (36500 ha^{-1}) and found the highest net production in T_3 which was the lowest stocking density.

Al hassan *et al.* (2012) carried out an experiment on the effects of stocking density on the growth and survival of *O. niloticus* cultured in hapa in a concrete tank. The authors were reported three different stocking densities as 56, 70 and 84 fish $hapa^{-1}$ which was designed as T_1, T_2 and T_3 respectively and observed the highest average weight gain in T_1 that stocking density was 56.

Kohinoor *et al.* (2012) reported an experiment of effects of stocking density on growth and production performance of indigenous stinging catfish, *H. fossilis* (bloch). The authors were carried out three different stocking densities of 1,25,000 ha^{-1} (T_1), 1,87,000 ha^{-1} (T_2) and 2,50,000 ha^{-1} (T_3) respectively and showed that highest growth and survival was found in T_1 that stocking density was 1,25,000.

Rahman *et al.* (2012) carried out an experiment on growth and survival of fingerlings of a threatened Snakehead, *Channa striatus* (Bloch) in earthen nursery ponds of Bangladesh. The authors were reported three different stocking densities of 150,000 ha^{-1} (T_1), 200,000 ha^{-1} (T_2), 250,000 ha^{-1} (T_3) and evaluated that higher survival of fingerlings was in T_1 where stocking density of 150,000 ha^{-1}.

Chakraborty and Banerjee (2010) evaluated an experiment on the effect of stocking density on monosex Nile tilapia growth during pond culture in India. The authors were noted different stocking densities as 5000, 10000, 15000, 20000, 25000 and 30000 fingerlings ha^{-1} and found that the highest growth rate was observed for the 20000 fingerling ha^{-1} density class which can be considered ideal for augmented production of the fish under Indian context.

Hasan *et al.* (2010) described an experiment on the effects of stocking density on growth and production of GIFT (*O. niloticus*) for a period of 100 days. Three stocking densities were tested 150, 200 and 250 fish dec^{-1}; designated as treatment T_1, T_2 and T_3 respectively. They were observed the best result in the treatment T_1 stocked with lowest stocking density (150 fish dec^{-1}).

Rahman and Marimuthu (2010) evaluated an experiment to assess the effect of different stocking density on growth, survival and production of native fish climbing perch (*A. testudineus*, Bloch) fingerlings in nursery ponds. The authors were carried out three different stocking densities at 1.0 million ha^{-1}, 1.2 million ha^{-1} and 1.4 million ha^{-1} and found that the highest growth, survival, production of the fingerlings of *A. testudineus* with three different densities in nursery ponds, 1.0 million ha^{-1}appears to be the best stocking density.

Farid *et al.* (2009) conducted an experiment on nursing and rearing of *Macrognathus aculeatus* at different stocking densities in pond culture system of Bangladesh. The authors were reported varying stocking densities and found to be better at a stocking density of 0.2 million fry $ha^{-1}$for achieving satisfactory growth performance and survival rate during the nursing stage.

Begum *et al.* (2008) reported an experiment on the effects of stocking density on growth and survival of *Mystus gulio* carried out in brakish water earthen nursery ponds. The authors were tested four different stocking densities of 200 m^{-2} (T_1), 250 m^{-2} (T_2), 350m^{-2} (T_3) and 450 m^{-2} (T_4) respectively and observed that 200-250 m^{-2} stocking density are best for the growth.

Kohinoor *et al.* (2007) stated an experiment of monoculture of climbing perch, Thai koi, *A. testudineus* (Bloch) under different stocking densities at on-farm in Bangladesh. The authors were described three different stocking densities such as 75,000 ha^{-1} (T_1), 100,500 ha^{-1} (T_2) and 125,000 ha^{-1} (T_3) respectively and observed that the highest growth in T_1 that the stocking density was 75,000.

Gokcek and Akyurt (2007) studied an experiment of the effect of stocking density on yield, growth and feed efficiency of Himri barbel (*Barbus luteus*) nursed in cages of Turkey. The authors were shown four different stocking densities at 40, 60, 80 and 100 fish per cage in 1m^3 and observed a highest mean weight was obtained in the lowest stocking density.

Chakraborty and Mirza (2007) worked an experiment of the effect of stocking density on survival and growth of endangered bata, *Labeo bata* (Hamilton–Buchanan) in nursery ponds. They described three different stocking densities at 0.6 million ha^{-1} (T_1), 0.80 million ha^{-1} (T_2), 1.0 million ha^{-1} (T_3) and found 0.6 million ha^{-1} appear to be most suitable stocking density for nursing and rearing of bata fry and fingerlings in single-stage nursery ponds.

Rahman *et al.* (2005) carried out an experiment on the effect of stocking density on survival and growth of critically endangered mahseer, *Tor putitora* (Hamilton), in nursery ponds. The authors were reported three different stocking densities at 0.6 million ha^{-1} (T_1), 0.8 million ha^{-1} (T_2), 1.0 million ha^{-1} (T_3) and showed that 0.6 million ha^{-1}appears to be the most suitable stocking density for rearing of mahseer fingerlings in single- stage nursery system.

Doolgindachbaporn *et al.* (2003) described an experiment on culturing of climbing perch, *A. testudineus* (Bloch) at varying densities. Climbing perch, with a mean weight of 0.198g, were cultured in 50 L circular tanks at densities of 55, 77, 99 and 121 fish m^{-2} respectively. The fish were fed floating pellet at 30% protein level for 16 weeks.

BFRI (1997) undertook stated an experiment on the culture of koi (*A. testudineus*) at a stocking density of 20,000 ha^{-1} to found the production potential of koi in earthen ponds. In the treatment of T_1, the gross production was 425 kg ha^{-1} in which rice bran 50%, mustard oil cake 30% and fish meal 20% were used as a supplementary feed. On the other hand, gross production of 286 kg ha^{-1} was obtained where rice bran 50% and mustard oil cake 50% were used as a supplementary feed (T_2). From that study it was concluded that the gross production of koi was significantly higher ($P<0.05$) in T_1 than of T_2.

Charoentesprasit *et al.* (1997) observed an experiment on climbing perch, *A. testudineus* and were stocked at three different stocking densities, 100, 200 and 300 fish 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 found optimum specific growth rate, length gain, weight gain, protein efficiency ratio, feed cost/unit weight gain and survival rate.

Akhteruzzaman (1988) showed an experiment on the monoculture of *A. testudineus* under semi-intensive culture system. Fingerlings of 8-9 g average weight were stocked at a density of 16,000 ha^{-1}. They were fed once per day with a mixture of rice bran, mustard oil cake and fishmeal at a ratio of 3:1:1. Feed was given daily at the rate of 5-6 % of the estimated body weight of the stocked fish. After 5 months rearing, the average yield was 450 kg ha^{-1} and the average survival rate was 78%.

Pathak (1978) studied an experiment with *A. testudineus* in two cemented cisterns for six months duration stocking with 300 juveniles (average wt. 1.05 g) in each. The author reported total production of 8.12 and 7.81 kg, respectively with 80% and 65% survivality and average weight attained by individual fish was around 33.83 and 40.05 g in six months.

2.2 Water quality parameter

Water quality parameters have a vital role in case of the nursing stage of the fish. These parameters are the most important factors which affect the aquatic production directly or indirectly. Some of the important parameters are noted below which are relevant to the research.

Kohinoor *et al.* (2016) observed that the average water temperature varied from 25.93 to 27.79 °C, transparency 30.51 to 33.55 cm, dissolved oxygen 5.15 to7.06 mgL^{-1}, pH 7.12 to 8.90, total alkalinity 108.21 to 143.05 mgL^{-1} and ammonia-nitrogen 0.05 to 0.92 mgL^{-1} during the investigation of water quality parameters in six farmers ponds situated in Kheruajani under Muktagacha upazilla, Mymensingh district, Bangladesh.

Habib *et al.* (2015) conducted an experiment on the physico-chemical parameters of water in nine earthen ponds containing Climbing perch (*A. testudineus*) of high yielding Vietnamese stock at the Fisheries and livestock farm of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The authors carried the mean values of various water quality parameters were, water temperature 26.7 to 31.1 °C, dissolved oxygen 4.90 to 7.20 mgL^{-1}, transparency 31.00 to 43.00 cm, pH 7.30 to 8.00, nitrate-nitrogen 0.05 to 0.21 mgL^{-1}, phosphate-phosphorous 0.11 to 2.0 mgL^{-1} and Ammonia-nitrogen 0.20 to 1.20 mgL^{-1}.

Monir and Rahman (2015) carried out an experiment in Freshwater sub-station, Bangladesh Fisheries Research Institute, Saidpur, Nilphamari of Bangladesh for a culture period of eight weeks. The authors found the water temperature ranged varied from 28 to 32 °C, transparency 30 to 58 cm, dissolved oxygen 2.8 to 6.5 mgL^{-1}, pH 7.6 to 8.6, total alkalinity 105 to 160 mgL^{-1} and ammonia – nitrogen (NH3-N) 0-0.07 mgL^{-1}.

Daudpota *et al.* (2014) conducted an experiment in fish hatchery Chilya Thatta, Sindh, Pakistan. The authors observed the water temperature ranged from 27.2 to 28.5 °C, dissolved oxygen 5.8 to 6.4 mgL^{-1}, pH 6.9 to 7.6, ammonia 0.45 to 0.51 mgL^{-1}, hardness 106 to 110 mgL^{-1} and nitrite 0.151 to 0.162 mgL^{-1}.

Rahman and Monir (2013) studied the physico-chemical parameters of nine nursery ponds situated at the Freshwater sub-station, Bangladesh Fisheries Research Institute, Saidpur, Nilphamari. The authors recorded the mean values of various water quality parameters were, water temperature 29 to 33 °C, transparency 31 to 58 cm, dissolved oxygen 3.4 to 6.2 mgL^{-1}, pH 6.8 to 8.5, total alkalinity 95 to 185 mgL^{-1} and NH_4-N 0 to 1 mgL^{-1}.

Asadujjaman *et al.* (2013) reported the physico-chemical parameters of nine similar sized ponds for a period of 90 days at the Fisheries Field Laboratory Complex, under the faculty of Fisheries, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh. The authors found the ranges of physico-chemical parameters as temperature 24.27 to 29.76 °C, transparency 44.38 to 45.62 cm, pH 7.86 to 9.13, dissolved oxygen 5.45 to 7.78 mgL^{-1}, total alkanity 50 to 200.52 mgL^{-1}, total ammonia 0.001 to 0.55 mgL^{-1}, nitrate-nitrogen (NO_3-N) 0.001 to 0.75 mgL^{-1} and phosphate-phosphorous (PO_4-P) 0.074 to 1.49 mgL^{-1}.

Al hassan *et al.* (2012) studied the physico-chemical parameters of three concrete tanks at the Fisheries Commission of the Ministry of Food and Agriculture (MOFA), Tamale in the Northern Region of Ghana. The authors showed the range of water quality parameters as temperature 31.1 to 31.8 °C, turbidity 34 to 57 cm, dissolved oxygen 2.53 to 3.65 mgL^{-1}, pH 6.2 to 7.2, ammonia 0.02 to 0.26 mgL^{-1} and nitrite .001 to 0.341 mgL^{-1}.

Kohinoor *et al.* (2012) measured the water quality parameters in nine farmer's ponds at Modhupur, Tarakanda, Mymensingh. The authors reported the mean values of water quality parameters such as, water temperature 23.88 to 30.70 °C, transparency 17.74 to 29.92 cm, dissolved oxygen 3.76 to 5.49 mgL^{-1}, pH 7.08 to 8.70, total alkalinity 95.04 to 133.29 mgL^{-1} and total ammonia 0.021 to 0.086 mgL^{-1}.

Chakraborty and Banerjee (2010) described the physico-chemical parameters at the fish farm in Rajarhat, North 24 parganas, West Bengal, India in 18 newly excavated 0.01 ha freshwater earthen ponds. They were found the water quality parameters values of temperature 31.0 to 32.7 °C, dissolved oxygen 7.1 to 7.4 mgL^{-1}, free CO_2 3.2 to 8.0 mgL^{-1}, pH 7.5 to 8.0, turbidity 28.2 to 40.0 cm and alkalinity 123.9 to 144.4 mgL^{-1}.

Rahman and Marimuthu (2010) stated the physico-chemical parameters of nine earthen nursery ponds containing Climbing perch (*A. testudineus*, Bloch) at the Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh. The authors observed the mean values of various water quality parameters as water temperature 27.60 to 31.00 °C, transparency 28.60 to 59.70 cm, dissolved oxygen 4.30 to 7.20 mgL^{-1}, pH 7.40 to 8.50, total alkalinity 82.50 to 171.50 mgL^{-1} and Ammonia-nitrogen 0.01 to 1.00 mgL^{-1}.

Mustafa *et al.* (2010) carried out an experiment related on the effect of stocking density on the physico-chemical parameters of water in 8 cemented tanks at Tongi Fish seed Multiplication Farm, Gazipur. The authors observed the ranges of physico-chemical parameters were as temperature 28.8 to 29.8 °C, pH 7.6 to 7.9 and dissolved oxygen 5.5 to 6.2 mgL^{-1}.

Hasan *et al.* (2010) conducted an experiment for a period of 100 days at "Nagla Fisheries Ltd.", Haluaghat, Mymensingh. The authors found that water temperature varied from 21.0 to 32.9 °C, dissolved oxygen 4.55 to 5.75 mgL^{-1}, pH 6.4 to 9.0, total alkalinity 82 to122 mgL^{-1} and transparency 17 to 29 cm.

Rahman *et al.* (2009) observed some physico-chemical properties of water in nine earthen nursery ponds containing critically endangered Reba Carp (*Cirrhinus ariza)* at the Freshwater Station, Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh. The authors reported the ranges of physico-chemical parameters as temperature 29.10 to 32.40 °C transparency 30.50 to 58.50 cm, pH 7.20 to 8.10, dissolved oxygen 3.10 to 6.00 mgL^{-1}, total alkalinity 65.50 to 171.50 mgL^{-1} and Ammonia-nitrogen 0.01 to 1.00 mgL^{-1}.

Farid *et al.* (2009) evaluated some physico-chemical properties of water in nine earthen ponds containing *Macrognathus aculeatus* in the field Laboratory complex of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. The authors stated the ranges of physico-chemical parameters as temperature 30.03 to 32.03 °C, transparency 25.30 to 26.67 cm, pH 7.5 to 8.07, dissolved oxygen 3.5 to 4.80 mgL^{-1}, alkanity 120.43 to 131.67 mgL^{-1}, Hardness 119.73 to 125.20 mgL^{-1}, Nitrate-nitrogen 1.33 to 2.20 mgL^{-1}, Nitrite-nitrogen 0.02 to 0.08 mgL^{-1}, ammonia-nitrogen 0.45 to 0.53 mgL^{-1} and phosphate-phosphorus 0.40 to 0.60 mgL^{-1}.

Begum *et al.* (2008) found that the average water temperature were varied from 22.29 to 28.62 °C, transparency 32.37 to 40.63 cm, pH 8.14 to 8.52, dissolved oxygen 4.61 to 7.55 mgL^{-1}, alkalinity 153.38 to 179.65 mgL^{-1}, nitrate-nitrogen (NO_3-N) 1.25 to 1.38 mgL^{-1} and phosphate-phosphorous (PO_4-P) 1.03 to 1.19 mgL^{-1} during the investigation of water quality parameters in twelve earthen ponds at the Bangladesh Fisheries Research Institute (BFRI), Brackish water Station, Paikgacha, Khulna.

Kohinoor *et al.* (2007) carried out an experiment on monoculture of climbing perch, Thai koi, *A. testudineus* (Bloch) under different stocking densities at on-farm. The authors observed the water temperature were ranged varied from 25.46 to 27.17 °C, transparency 24.53 to 33.38 cm, pH 7.05 to 8.20, dissolved oxygen 4.70 to 6.80 mgL^{-1} and total alkalinity 113.9 to 146.2 mgL^{-1}.

Alim (2005) showed the water temperature to range from 17.30 to 33.50 °C, transparency from 17 to 60 cm, pH from 6.2 to 8.9, dissolved oxygen from 1.2 to 8.50 mgL^{-1} from the pond situated in the Bangladesh Agricultural University campus, Mymensingh.

Uddin (2002) carried out an experiment in earthen ponds at the Field laboratory Bangladesh Agricultural University, Mymensingh. The author found the temperature varied from 25.6 to 33.0 °C, transparency 11.0 to 63.5 cm, DO 2.2 to 8.8 mgL^{-1}, pH 6.14 to 8.88, NO_3-N 0.01 to 0.88 mgL^{-1}, PO_4-P 0.03 to 4.46 mgL^{-1} and total alkanity 45 to180 mgL^{-1}.

Sarker (2000) concluded an experiment in six ponds on the effect of periphyton to the monoculture of Gift tilapia situated at the southeast corner of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. During the study period the author observed the water temperature varied from 19.8 to 22.8 °C, transparency 27 to 35 cm, pH 6.8 to 8.3, dissolved oxygen 3.75 to 8.84 mgL^{-1}, phosphate- phosphorous (PO_4-P) 0.49 to 4.07 mgL^{-1} and nitrate- nitrogen (NO_3-N) 1.69 to 2.6 mgL^{-1}.

Haque (2000) studied the average water temperature were varied from 29 to 32 °C, transparency 30 to 65 cm, dissolved oxygen 3.5 to 7.8 mgL^{-1}, pH 6.8 to 7.8, nitrate-nitrogen (NO_3-N) 1.64 to 2.31 $mgL^{-1}$and phosphate-phosphorous (PO_4-P) 0.31 to 1.07 mgL^{-1} during the investigation of water quality parameters in six ponds situated at the south-east corner of the Fisheries Faculty Building, Bangladesh Agricultural University, Mymensingh.

Rahman (2000) conducted a research work in ten earthen ponds at the Field Laboratory of Bangladesh Agricultural University, Mymensingh and showed that temperature were ranged from 21.10 to 32.2 °C, transparency 12 to 41 cm and alkanity 37 to 151 mgL^{-1}.

Kohinoor *et al.* (1998) observed that the water quality parameters in six research ponds at Field Laboratory Complex, Faculty of Fisheries, Bangladesh Agriculture University, Mymensingh. The authors stated the mean values of water quality parameters such as, water temperature (°C), transparency (cm), pH dissolved oxygen (mgL^{-1}), total hardness (mgL^{-1}) and ammonia nitrogen (mgL^{-1}) were 27.72±0.01, 32.58±2.401, 7.18±0.06, 4.20±0.12, 104.81±8.12 and 0.12±0.02 respectively.

Hasan (1998) evaluated the water quality parameters in 15 experimental ponds at the Field Laboratory Complex, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. The author observed the mean values of water temperature (°C) 28.54, transparency (cm) 30.64, total ammonia (mgL^{-1}) 0.29, total hardness (mgL^{-1}) 137.56, dissolved oxygen (mgL^{-1}) 3.91, phosphate-phosphorous (mgL^{-1}) 0.48 and nitrate-nitrogen (mgL^{-1}) 1.68.

Paul (1998) carried out an experiment on the water quality parameters in six ponds of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh and found the various water quality parameters such as water temperature 26.7 to 33.7 °C, pH 6.51 to 9.45, DO 0.85 to 7.8 mgL^{-1} and alkalinity 70 to 210 mgL^{-1} during the research period.

Begum (1998) described the some water quality parameters and found temperature ranged from 25.1 to 33.1 °C and DO from 5.06 to 7.09 mgL^{-1} in fish ponds of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, for the time span August to October, 1997.

Nirod (1997) carried out an experiment on the water quality parameters in nine research ponds of Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. The author found the following range of physico-chemical parameters such as temperature 21.8 to 34.10 °C, transparency 25 to 27 cm, pH 6.5 to 8.5 and DO 3.40 to 8.79 mgL^{-1}.

DoF (1996) recorded that the range of DO for suitable water body for fish culture would be 5-8 mgL^{-1} and for pH 6.5-8.0.

2.3 Dietary protein requirement

Ramadan and Sief (2006) conducted an experiment on the effects of stocking density and protein level in diet on growth performance, survival rate and feed efficiency of Nile Tilapia *(Oreochromis niloticus*) fry monosex during the nursery period. The authors reported two stocking densities 1389 and 2778 hatchlings m^{-3} within each density 29.71, 34.43 and 39.11% dietary protein levels and observed the best specific growth rate and final weight in lowest stocking density 1389 hatchlings m^{-3} and 39.11% protein level.

Hakim *et al.* (2001) stated the effect of protein level and stocking density on growth performance of Nile tilapia (Oreochromis niloticus) cultured in tanks. The authors reported two stocking densities 50 and 100 fish m^{-3} and two protein level 25% and 30% and observed increasing dietary protein level fed improved the specific growth rate regardless of stocking density.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

This experiment was designed to fulfill the objectives of this current study. For this experiment, study was planned to determine the effect of stocking density on growth, survival and production of Thai koi fry in the nursery ponds.

3.2 Study area

The experiment was carried out in the Nilphamari district under Saidpur upazilla (Figure 3.1) which located at 25° 46.7′ N and 88° 53.5′ E of Bangladesh. The study area was selected based on the following reasons:

- Availability of seasonal ponds
- Farmer’s were more interested
- Well- based communication system.

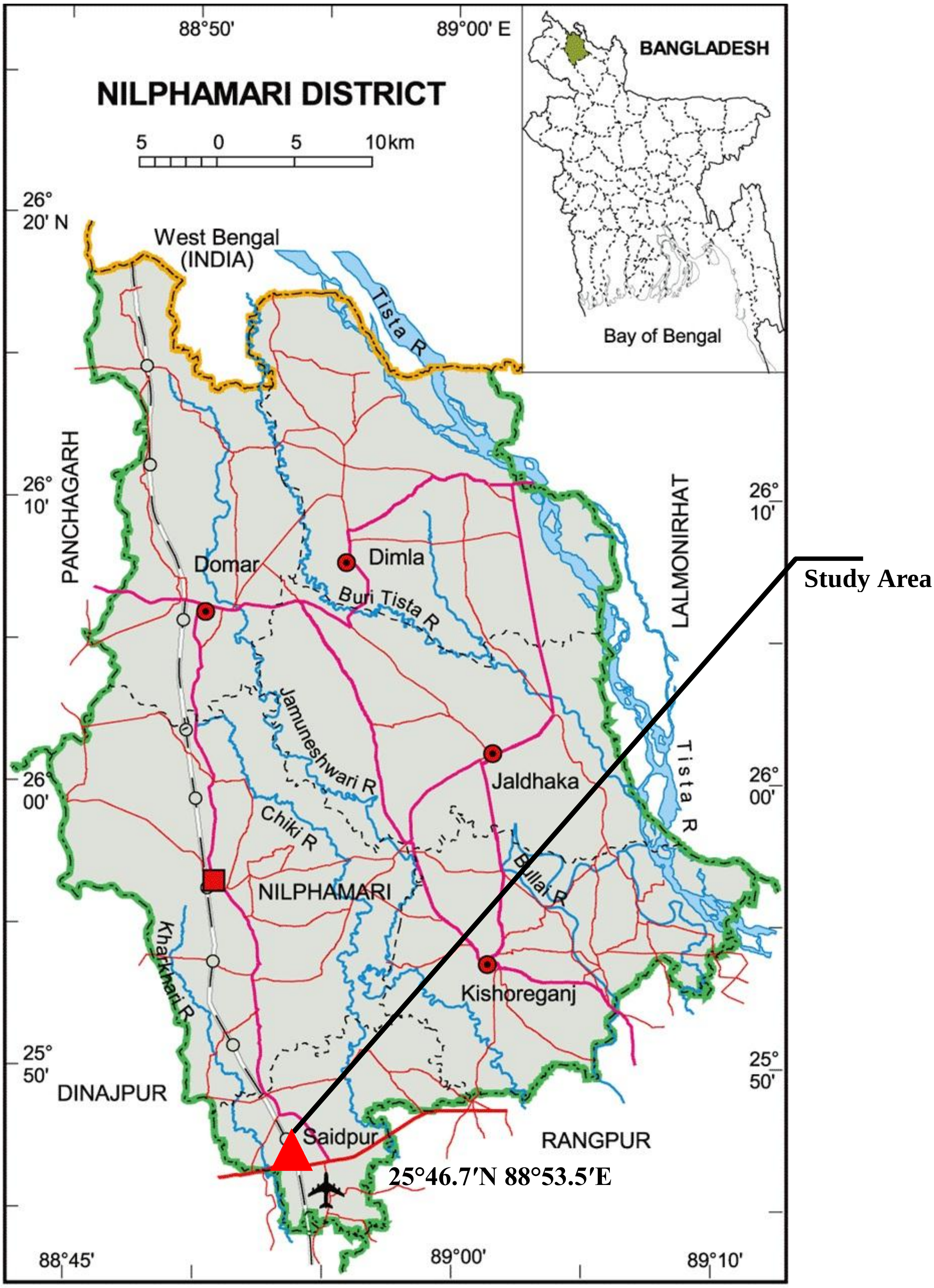

Figure 3.1 Map showing the study area.

3.3 Experimental period

The study was conducted for a period of 30 days from 15thApril to15th May 2016.

3.4 Description of the Experimental units

Nine earthen ponds were selected at Nilphamari district under Saidpur upazilla for the experimental purpose. The ponds were rectangular, semi-rectangular in shape and size was between 15-20 decimal in which the average water depth of each pond was 1.52 meter. In order to proper management and protect from poaching ponds were selected near the farmer house. The ponds were well exposed to sunlight and were not interconnected by inlet and outlet. Although the main source of water was the rainfall, in some cases water was supplied from a deep tube-well using a flexible plastic pipe whenever needed. The embankment was well protected and covered with grass.

3.5 Design of experiment

The experiment was undertaken 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 Thai koi fry were 1200, 1400 and 1600 dec^{-1} which were designed as T_1, T_2 and T_3 respectively.

3.6 Pond preparation

3.6.1 Control of undesirable species and aquatic weeds

Before commencing the experiment all ponds were renovated and aquatic vegetations were removed manually. The weeds of embankment were cleaned manually and pond dikes were repaired by using the excavated bottom soils. Unwanted fishes and other aquatic organisms were eradicated by the application of rotenone at the rate of 30-40 gm dec^{-1} $meter^{-1}$. The dead fishes and other aquatic organisms were then removed by repeated netting.

3.6.2 Liming

Lime (CaO) was applied at a rate of 1 kg dec^{-1} after one week of rotenone application. Lime was liquefied into an earthen pot and then applied by spreading homogenously in the ponds.

3.6.3 Fertilization

After 4 to 5 days of liming, ponds were fertilized using urea and TSP each at a rate of 100g dec^{-1} and 200 g dec^{-1} respectively. TSP was spread all over the ponds after 10 to 12 hours dissolving in plastic buckets.

3.7 Experimental Fish

The fry of Thai koi (*A. testudineus*) was selected for the experimental purpose. The Thai koi fry of an average weight of 0.1g were brought from a commercial fish hatchery (Sondhi Hatchery) located in the Taragonj upazila of Rangpur district and transported to the BFRI sub-station using oxygenated plastic bags filled with freshwater. Prior to the commencement of the study, the experimental fish were acclimatized for 7 days in the pond of BFRI sub-station, Saidpur. Thereafter, fish with an average weight of 0.2 g were stocked (Plate 3.1) at the densities of 1200, 1400 and 1600 fry $dec^{-1}$of pond which represented as T_1, T_2 and T_3 respectively.

Plate 3.1 Stocking of fish in the experimental pond.

3.8 Feeding

Feeding rate was maintained according to the body weight of the experimental fish. All the experimental fish were fed with a commercial feed containing 35.53% crude protein (nursery Lily fish feed) three times a day at a rate of 25% body weight for the first 10 days, 15% for the second 10 days, and finally 10% for the last 10 days. The feed was supplied evenly all over the pond's surface three times a day at 07:00 am, 12.00 pm and 5:00 pm.

3.9 Monitoring and data collection

The survivals of fish were recorded throughout the nursing period. Sampling was done for the calculation of feeding rate in every ten days intervals using a net. For monitoring the growth and to regulate the amount of feed to be given, the bulk weight of around 10% sampled fish were taken from each pond. The weight of Thai koi was measured by using a digital electronic balance (TANITA KD160, Plate 3.2). During the experimental period, all the ponds were visited regularly for monitoring the pond conditions. The sampled fish were handled very carefully in order to reduce the handling stress.

Plate 3.2 Measuring weight (g) of Thai koi during the study period.

3.10 Water quality assessment

3.10.1 Procedure of the study

In the present study, water sample were collected from each ponds. Recording on the spot data and collection of samples were made between 9.00 to 11.30 A.M. Water temperatures, transparency, pH, dissolved oxygen (DO), total alkalinity, and total hardness were recorded every 10 days interval between 9.00 am to 11.30 am.

3.10.1.1 Temperature

Temperature of water was taken from each pond by using a standard mercury thermometer (Plate 3.3).

Plate 3.3 Measurement of water temperature during the experimental period.

3.10.1.2 Transparency

Transparency of water was taken from each pond by using a secchi disc.

3.10.1.3 pH

pH of water was taken from each pond by using a digital pH meter (Elico-Li-120) (Plate 3.4).

Plate 3.4 Measurement of pH during the experimental period**.**

3.10.1.4 Dissolved Oxygen

The dissolved oxygen was measured by using digital DO meter (YSL, Model 58, and USA).

3.10.1.5 Total Alkalinity

The total alkalinity was measured by following the 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

Total hardness was measured by applying EDTA and HI: 3812-0 solution using Hardness Test kit HI 3812 as described in Appendix A.2 (Plate 3.5).

Plate 3.5 Determination of total hardness during the experimental period.

3.10.1.7 Ammonia

The ammonia content of water was measured using an ammonia test kit for freshwater (Hanna, Romania) as mentioned detailed in Appendix A.3.

3.11 Harvesting

Fishes were completely harvested after 30 days of nursing, Harvesting of fishes were performed by repeated netting using a net (Plate 3.6) and dewatering of the ponds. During harvesting, the weights of all fish were also taken.

Plate 3.6 Harvesting of fish at the end of the experiment.

3.12 Growth performance and production of fish

The following growth performances and survival were evaluated using the following equations:

(i) Growth

a) Weight gain (g)

Weight gain (g) = Final weight (g) –Initial weight (g)

b) % Weight gain

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

c) 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:

$$\text{SGR}\ (\%\ \text{day}^{-1}) = \frac{\text{In}\ w_2 - \text{In}\ w_1}{T_2 - T_1} \times 100$$

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).

d) Survival rate (%)

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

ii) Feed conversion ratio (FCR)

Feed conversion ratio is defined as the amount of dry feed intake per unit live weight gain. It is calculated as:

$$\text{FCR} = \frac{\text{Total dry feed intake (g)}}{\text{Total wet weight gain (g)}}$$

To calculate FCR, the dry weight of the feed was obtained by using a correction for the analyzed moisture content of the diet. FCR is a measure of the degree of gross utilization of feed for growth.

iii) Protein utilization

Protein efficiency ratio (PER)

Protein efficiency ratio is defined as the gain in weight of fish per gram of crude protein fed. Protein efficiency ratio is calculated as:

$$\text{PER} = \frac{\text{Wet weight gain (g)}}{\text{Total protein intake (g)}} \times 100$$

PER gives an indication of the efficiency of utilization of dietary protein.

3.13 Production of fishes

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

3.14 Proximate composition analysis

Sampling procedure

At the end of the experiment, five fish were then randomly selected from each replicate (fifteen fish per treatment) and brought to the laboratory for the determination of whole body proximate composition. The selected fish were killed by keeping them in a refrigerator. After being killed, the individual fish were weighed immediately and kept in an oven for drying. Thereafter, the dried fish were then grinned using a blender machine in order to determine the moisture, crude protein, crude lipid and ash content of whole fish body.

Whole fish body composition was determined by using the standard protocols as mentioned by the Association of Official Analytical Chemists (AOAC, 1997).

3.14.1 Moisture

The moisture content of the sample was analyzed by drying the samples in the Hot Air Oven at 105 ℃ for 12 hours until constant weight was obtained as explained in detailed in Appendix B.1. The loss of weight was calculated as percent moisture. The percent moisture content was calculated by applying the following equation (Bhattacharya, 2013):

% of moisture = E/C x 100

Where,

E= Weight of moisture. C= Weight of sample.

The moisture free samples were then used in order to determine the crude protein, lipid and ash content.

3.14.2 Crude protein

The crude protein content of the fish samples was calculated indirectly by determining the total nitrogen content of the sampled of the fish by a Kjeldahl method using kjeldhal apparatus. In this case, total nitrogen content was determined by digesting the sample with concentrated sulphuric acid (H_2SO_4), in presence of digestion mixture into boric acid as explained in detail in Appendix B.2. The total nitrogen value was then calculated by using 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

The 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 thereafter the solvent was evaporated by heating in the oven at 80 °C as explained in detail in Appendix B.3. The Lipid content of the fish samples was then determined by using 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

Muffle furnace, desiccator and an electronic balance were used to determine the ash content of the fish sample. The moisture free samples were taken in porceline basin made crucible and weighed. Thereafter the ash content was measured by igniting the samples in a muffle furnace at a temperature of 550 °C for 6 hours. The samples were then cooled in a desiccator. The average weight of each sample of the remaining material was taken as percentage as mentioned in Appendix B.4. Ash content of the whole fish body was then calculated 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 Economic analysis

Gross margin (BDT treatment $^{-1}$) = Gross return – Gross variable costs

$$\text{BCR} = \frac{\text{Gross return}}{\text{Gross variable costs (BDT)}}$$

3.16 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 differences between means. The data are expressed as average ± SE and statistical analysis was performed using SPSS version 20 and Microsoft Office Excel for Window.

CHAPTER 4

RESULTS

4.1 Water quality Parameters

Mean values of water quality parameters over the period of Thai koi nursing are presented in Table 4.1. Generally with the exception of water temperature and pH and all the other water quality parameters showed significant (*P<0.05*) variation among the treatments. The results showed that the highest temperature of 27.5 °C was recorded for treatments T_3 while the least temperature of 27.3 °C was observed in treatments T_1 and T_2. However, there was no significant difference in the water temperature recorded among the treatments. While, a significantly highest (*P<0.05*) water transparency was observed in the treatment of T_3 compared to all the remaining treatments and the mean values were as 27.66±0.34, 30.01±0.58, 32.83±0.41 cm in T_1, T_2 and T_3 respectively.

In the case of pH, a non-significantly higher (*P>0.05*) pH was noted in T_3 (7.87±0.51) than T_1 (7.35±0.58) and T_2 (7.64±0.59). A significantly lower (*P<0.05*) content of dissolved oxygen (mgL^{-1}) was observed in T_3 compared to the treatment of T_1 and T_2.The mean values of dissolved oxygen content in the experimental pond 5.16±0.01, 5.15±0.01 and 5.03±0.01 mgL^{-1} in T_1, T_2 and T_3 respectively.

Usually an increasing trend of total alkalinity and hardness were observed with the increasing of stocking density of Thai koi. The highest total alkalinity of 108.37 mgL^{-1} was recorded in T_3 while the least total alkalinity of 100.23 mgL^{-1} was observed in T_1. There was significant (*P<0.05*) difference in the total alkalinity noted among the treatments. The Hardness values (mgL^{-1}) in all treatments were differed significantly

($P<0.05$), while a comparatively lower level of Hardness was recorded in the treatment of T_1.

Unlike total alkalinity and hardness, the ammonia content was also showed an increasing pattern with the increasing of stocking density. The mean values of ammonia-nitrogen (unionized) were 0.390±0.01, 0.420±0.01, 0.423±0.00 in $T_{1,}$ T_2 and T_3 respectively. However, the significantly lowest ($P<0.05$) ammonia content was noted in the treatment of T_1 when compared with the remaining two treatments.

Table 4.1 Water quality parameters of experimental pond during the study period

Water quality parameters	**Treatments**		
	T_1	**T_2**	**T_3**
Temperature (°C)	$27.30±0.06^{a}$ (27.20-27.40)	$27.30±0.06^{a}$ (27.20-27.40)	$27.50±0.06^{a}$ (27.40-27.60)
Transparency (cm)	$27.66±0.34^{a}$ (26.98-28.00)	$30.01±0.58^{b}$ (29.00-31.02)	$32.83±0.41^{c}$ (32.10-33.50)
pH	$7.35±0.58^{a}$ (6.34-8.36)	$7.64±0.59^{a}$ (6.62-8.66)	$7.87±0.51^{a}$ (6.95-8.70)
Dissolved Oxygen (mgL^{-1})	$5.16±0.01^{b}$ (5.15-5.17)	$5.15±0.01^{b}$ (5.14-5.16)	$5.03±0.01^{a}$ (5.02-5.05)
Alkalinity (mgL^{-1})	$100.23±0.52^{a}$ (99.40-101.20)	$103.70±0.55^{b}$ (102.80-104.70)	$108.37±0.38^{c}$ (107.80-109.10)
Hardness (mgL^{-1})	$39.77±0.55^{a}$ (38.80-40.70)	$49.20±0.30^{b}$ (48.60-49.50)	$75.70±0.52^{c}$ (74.80-76.60)
Ammonia (mgL^{-1})	$0.39±0.01^{a}$ (0.38-0.40)	$0.42±0.01^{b}$ (0.41-0.43)	$0.43±0.00^{b}$ (0.42-0.44)

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

4.2 Growth performance

The growth performances, feed utilization and survival of Thai koi (*A. testudineus*) fry after 30 days rearing is given in table 4.2. Growth of Thai koi in nursing ponds was investigated and the results revealed that the growth rates were varied according to their stocking densities. On the basis of final mean weight attained under T_1, T_2 and T_3 were 6.57±.90g, 4.49±.23g and 3.70±.23g respectively. The results showed that significantly ($P<0.05$) highest weight was obtained in $T_{1,}$ while the lowest was found in the case of T_3. Therefore, the results revealed that the higher growth performance was noted at the lowest stocking densities. The highest weight gain was obtained in T_1 and lowest in T_3. The mean weight gain showed significant differences ($P<0.05$) in T_1 followed by T_2 and T_3. The specific growth rate (% day^{-1}) of fish in different treatments were varied among the treatments. The highest value of specific growth rate (SGR) was 11.26±0.41 in T_1 and the lowest was 9.66±0.25 in T_3. The SGR in T_1 was significantly ($P<0.05$) higher than T_3, while T_2 did not differed significantly ($P>0.05$) from $T_{1.}$

Feed utilization parameters such as FCR and PER were also evaluated at the end of feeding trial. Generally, FCR showed a decreasing trend while PER showed an increasing trend with the increasing of stocking density of Thai koi. The FCR value of T_1, T_2 and T_3 were noted as 0.64, 0.71 and 1.11 respectively, while the FCR value of T_1 was observed to be significantly ($P<0.05$) lowest which indicates that lower amounts of feed needed to produce one unit fish biomass and the highest was obtained in $T_{3.}$ Therefore, FCR was best for fish in T_1 where the lowest number of fry was stocked (1200 dec^{-1}). On the other hand the PER was highest in T_1 followed by T_2 and T_3 respectively. The survivality of Thai koi as percentage was also recorded through-out the culture periods which were 86.67±1.66, 85.00±2.89, and 70.00±0.58 for T_1, T_2 and T_3

respectively, while the highest survival rate was observed in T_1 and the lowest in T_3. Differences in survival rates among the treatments were found to be significant ($P<0.05$).

Table 4.2 Growth performances, feed utilization and survival of Thai koi (*A. testudineus*) fry after 30 days rearing

Parameters	Treatments		
	T_1 (1200 frydec^{-1})	**T_2 (1400 fry dec^{-1})**	**T_3 (1600 fry dec^{-1})**
Initial Weight (g)	0.22 ± 0.01^{a}	0.22 ± 0.01^{a}	0.20 ± 0.00^{a}
Final Weight (g)	6.57 ± 0.90^{b}	4.49 ± 0.23^{a}	3.70 ± 0.23^{a}
Weight gain (g)	6.35 ± 0.90^{b}	4.27 ± 0.23^{a}	3.50 ± 0.24^{a}
Specific growth rate (SGR) (% day^{-1})	11.26 ± 0.41^{b}	10.10 ± 0.29^{a}	9.66 ± 0.25^{a}
Feed conversion ratio (FCR)	0.64 ± 0.09^{a}	0.71 ± 0.09^{a}	1.10 ± 0.06^{b}
Protein efficiency ratio (PER)	4.59 ± 0.71^{b}	4.10 ± 0.45^{ab}	2.59 ± 0.15^{a}
Survival rate (%)	86.67 ± 1.67^{b}	85.00 ± 2.90^{b}	70.00 ± 0.58^{a}

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

4.3 Net production

The total productions of Thai Koi in the three treatments over 30 days rearing in farmer's seasonal nursery ponds are shown in Figure 4.1. The mean productions of *A. testudineus* were 6.83±0.91, 5.36±0.43 and 4.14±0.29 Kg dec^{-1} in the treatments of T_1, T_2 and T_3 respectively. The better production was noted 6.83 Kg dec^{-1} (T_1) and the lowest production was observed 4.14 Kg dec^{-1} (T_3). The total production of *A. testudineus* in T_1 differed significantly ($P<0.05$) with the T_3.

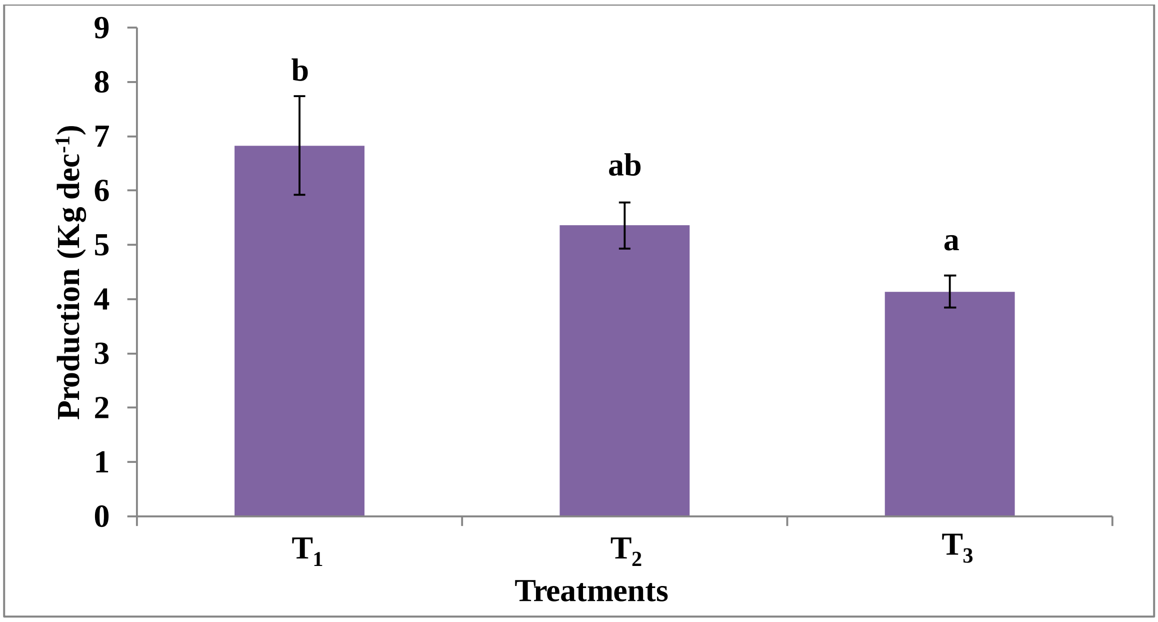

Figure 4.1 Total production of Thai koi in different treatments (kg dec^{-1}) after 30 days rearing.

4.4 Proximate Composition

Data on the whole body composition of Thai koi Shown in Table 4.3. No effect ($P>0.05$) was observed on the whole body moisture, lipid and ash content and showed only a significant effect in the whole body protein content. The high moisture content was observed in T_1 while the lowest moisture content was observed in T_3. The maximum protein content of 11.30±0.33 was recorded in T_1 while the least amount of protein 9.47±0.30 was observed in T_3. In case of lipid, the highest amount was found in T_1 and the lowest amount was noted in T_3. On the other hand, the high ash content was observed in the treatment of T_3 while the lowest in the case of T_1.

Table 4.3 Whole body proximate composition of Thai koi (*A. testudineus*) after 30 days rearing

Parameters	Treatments		
	T_1	**T_2**	**T_3**
Moisture (%)	78.39 ± 0.48^a	78.04 ± 0.60^a	76.79 ± 0.32^a
Protein (%)	11.30 ± 0.33^b	9.82 ± 0.48^a	9.47 ± 0.30^a
Lipid (%)	5.39 ± 0.53^a	5.10 ± 0.59^a	5.07 ± 0.55^a
Ash (%)	4.27 ± 0.58^a	4.68 ± 0.69^a	4.80 ± 0.54^a

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

4.5 Economic analysis

A simple economic analysis was conducted to evaluate the net profit from this nursing operation. The total production of Thai koi as recorded in T_1, T_2 and T_3 were 6.83±0.91, 5.36±0.43 and 4.14±0.29 kg dec^{-1} respectively. The production of fish was highest in T_1 but did not vary significantly with T_2. Therefore the lowest variable cost (BDT dec^{-1}) was recorded in T_1 (1172.27±94.08) in those of T_2 (1232.95±13.85) and T_3 (1356.01±10.24) (Table 4.4). In addition, constantly highest gross margin (BDT dec^{-1}) was found in T_1 (1147.06±68.40) over T_2 (907.73±121.08) and T_3 (883.69±26.43) together with highest BCR was recorded in T_1 (2.04±0.07) followed by T_2 (1.80±0.15) and T_3 (1.65±0.03) (Table 4.4).

Table 4.4 Benefit and cost analysis of Thai koi (*A. testudineus* dec^{-1}) nursing of the experimental ponds for a period of 30 days

Item wise expenditures/operational cost	**Treatments**		
	T_1	**T_2**	**T_3**
Pond preparation	1500.00	1500.00	1500.00
Price of fry (BDT $treatment^{-1}$)	11000.00	12600.00	15466.00
Lime, fertilizer(BDT $treat^{-1}$)	1000.00	1000.00	1000.00
Feed costs (BDT $treat^{-1}$)	4063.75	4678.70	5242.16
Transport, labor etc. (BDT $treat^{-1}$)	3000.00	3000.00	3000.00
Gross variable costs(BDT dec^{-1})	1172.27 ± 94.08^{a}	1232.95 ± 13.85^{a}	1356.01 ± 10.24^{a}
Income and output			
Total production(kg dec^{-1})	6.83 ± 0.91^{b}	5.36 ± 0.43^{ab}	4.14 ± 0.29^{a}
Gross return (BDT dec^{-1})	2380 ± 80.83^{b}	2240 ± 18.48^{ab}	2080 ± 40.00^{a}
Gross margin (BDT dec^{-1})	1147.06 ± 68.40^{a}	907.73 ± 121.08^{a}	883.69 ± 26.43^{a}
Benefit Cost Ratio (BCR)	2.04 ± 0.07^{b}	1.80 ± 0.15^{ab}	1.65 ± 0.03^{a}

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

CHAPTER 5

DISCUSSION

Growth, feed efficiency and feed consumption of fish are normally governed by few environmental factors (Fry, 1971). Environmental factors exert an immense influence on the maintenance of a healthy aquatic environment and production of food organism. Among the environmental factors, water quality parameters play a significant role in the culture of fish and other aquatic organisms. Good water quality is undoubtedly a prerequisite for fish growth and their survival. The water quality parameters were measured in the present study throughout the experimental period which showed a significant variation among the treatments. However, all of those parameters were within the acceptable range for fish culture (Jhingran, 1991).

Temperature plays a vital role in respect of fish production. In the present study, the average temperature was recorded 27.3±0.06, 27.3±0.06, 27.5±0.06 °C in T_1, T_2 and T_3 respectively. Previously, several studies showed that 25 to 35 °C (Aminul, 1996), 27.60 to 31.00 °C (Rahman and Marimuth, 2010), 26.80 to 31.80 °C (Kohinoor *et al.* 2016) and 26.93 to 27.41 °C (Roy *et al.* 2002) are suitable for fish culture. There was no significant variation of temperature among all the treatment groups and those values were within the acceptable ranges for fish culture.

Water transparency in the current study was significantly higher at high stocking density and consistently lower at low density, which might be due to the reduction of plankton production of high density of fish (Rahman and Monir 2013). During the experimental period the average transparency level were 27.66±0.34, 30.01±0.58 and 32.83±0.41 cm in T_1, T_2 and T_3 respectively. Lowest transparency was recorded in T_1, which might be because of the increase of the plankton population density due to lower density of fish

(Haque *et al*. 1994 and Rahman *et al.* 2005). Kohinoor *et al.* (2016) found that the transparency content lies between 26.75 to 30.44 cm, while Ahmed *et al.* (2009) recorded 22 to 33 cm which is more or less similar to the present study. Therefore, it can be said that the water transparency in the current experimental pond is suitable for fish culture (Boyd, 1982).

In addition, pH in the water body absolutely is an important factor for successful fish culture. Abrupt change of pH in the culture system may hamper the production alarmingly. In the current study, the average values of pH in the treatments were 7.35±0.58, 7.64±0.59 and 7.87±0.51 in T_1, T_2 and T_3 respectively. According to Boyd (1992) pH 6.5 to 8.5 are suitable for fish culture. Rahman and Marimuth (2010) also recorded that the suitable range of pH value is 7.40-8.50 for endangered native fish climbing perch; 6.5 to 8.1 for benthic fauna (Shariful *et al.* 2009) and 0.03 to 9.03 carp SIS polyculture (Roy *et al.* 2002). From this context, it can be said that the experimental ponds were suitable for fish culture.

Usually successful fish culture depends on the careful management of dissolved oxygen at optimum level. DoF (1996) reported that the range of dissolved oxygen content for fish culture should be 5.0-8.0 mgL^{-1}. The suitable range of dissolved oxygen can be very depending on the species being cultured. Kohinoor *et al.* (2012) observed the dissolved oxygen content between 4.23-5.32 mgL^{-1} are acceptable for indigenous stinging catfish (*H. fossilis*) culture, while 4.13 to 4.71 $mgL^{-1}$are sufficient for nursing of Thai *koi* (*A. testudineus*) (Rahman *et al.* 2013). However, according to Wahab *et al.* (1995) dissolved oxygen content of a productive pond should not be less than 4 mgL^{-1}. The average dissolved oxygen levels in the present study were 5.16±0.01, 5.15±0.01 and 5.03±0.01

mgL^{-1} in T_1, T_2 and T_3 respectively. Therefore, the dissolve oxygen content in the present study was acceptable for fish culture.

Total alkalinity has little direct effect on fish growth, however the well-being of fish may be indirectly affected by this parameter, because water of low values of alkalinity are generally biologically less productive than those with high values. According to Alikunhi (1957) total alkalinity more than 100 mgL^{-1} should be present in highly productive water bodies, while Boyd (1982) reported that the total alkalinity should be more than 20 mgL^{-1} in any fertilized ponds as production increases with the increase of total alkalinity. During the present study, the average alkalinity values were 100.23±0.52, 103.70±0.55 and 108.37±0.38 mgL^{-1} respectively in T_1, T_2 and T_3. These values were more or less similar to the results of (Rahman and Marimuth, 2010; Kohinoor *et al.* 2016 and Roy *et al.* 2002). Therefore, it might be concluded that the total alkalinity content of this experiment was suitable for Thai koi nursing.

However, ammonia concentration was found to vary from 0.38 to 0.40, 0.41 to 0.43 and 0.42 to 0.43 mgL^{-1} during the experimental period in T_1, T_2 and T_3 respectively. The highest amount of ammonia was found in the treatment of T_3, which might be due to the release of higher amounts of faecal materials in the ponds of higher number of fish (Ahmed *et al.* 2017). The ammonia content may vary from 0.20 to 0.57 mgL^{-1} for Nile tilapia and freshwater prawn culture (Rahman, 2005) and 0.01 to 0.82 mgL^{-1} for freshwater prawn post larvae nursing (Asaduzzaman *et al.* 2006). Increase in stocking density may also cause the deterioration in water quality, resulting in stressful conditions (Barton and Iwama, 1991 and Pankhurst and Kraak, 1997). This current study demonstrated that the lowest stocking density showed a comparatively lower amount of ammonia, which might be a reason for the best growth performance and feed utilization parameter in the treatment of T_1.

Stocking density is a very vital parameter which may affect directly the growth of fish and its production (Backiel and Le Cren, 1978). The results of this study clearly demonstrate that the stocking density significantly affected growth rate, feed utilization and production of *A. testudineus* fry reared at different stocking density in seasonal nursery ponds.

Growth in terms of weight gain of fingerlings of *A. testudineus* was significantly higher in T_1 where the stocking density (1200 dec^{-1}) was low compared to those of T_2 (1400 dec^{-1}) and T_3 (1600 dec^{-1}) although same feed was applied at an equal ratio in all the treatments. This is might be due to competition for feed and habitat because of the higher number of fish (Rahman and Marimuthu, 2010). Similar findings also have been reported in *M. gulio* (Begum *et al.* 2008), *A. testudineus* (Rahman and Marimuthu, 2010) and *O. niloticus* (Mensah *et al.* 2013).

In the present study, the specific growth rate (% day^{-1}) was found higher in the treatment of T_1 (11.26) compared to T_2 (10.10) and T_3 (9.66). The highest specific growth rate was found in the lowest stocking density, while the lowest specific growth rate was observed in the highest stocking density. Similar findings were also noted in Mirror carp, Calbasu, Indian and Chinese carps and Thai koi (Haque *et al.* 1994; Rahman *et al.* 2004, 2005 and Kohinoor *et al.* 2007).The higher specific growth rate in the lowest stocking density is might be due to availability and better utilization of food.

However, significantly lowest FCR was observed in T_1 followed by T_2 and T_3. The FCR values of the present study are lower than those reported by many workers (Reddy and Katro, 1979; Das and Ray, 1989; Islam, 2002; Islam *et al.* 2002 and Rahman *et al.* 2005), which might be because of the proper utilization of feed for smaller ration size and higher digestibility. Increasing trend of FCR values were observed with increasing ration size that were fed to Indian major carp and air-breathing catfish (Das and Ray, 1989) and common carp (*C. carpio*) fed with supplementary feed (Ghosh *et al.*1984). De Silva and Davy (1992) stated that digestibility plays an important role in lowering the FCR value of efficient utilization of food. The digestibility in turn depends on many factors such as daily feeding rate, its frequency, and the type of feed used (Chiu *et al.* 1987). However, the lower FCR value in the present study indicates better food utilization efficiency, despite the values increased with increasing stocking densities.

In addition, PER gives an indication of the efficiency of utilization of dietary protein. In the present study the FCR and PER values were found to show an inverse relationship at different stocking densities. The highest PER values found in the lowest stocking density while the lowest PER values in the highest stocking density. In contrast to the present findings the highest PER value was found in the case of medium stocking density (Habib *et al.* 2015).

The significantly higher survival rate of fingerlings was obtained in T_1, where the stocking density was the lowest compared to those in T_2 and T_3. The causes for decreasing the survival rates in the higher stocking density were accounted for higher stocking density of fingerlings as well as competition for food and space in the experimental ponds (Rahman *et al.* 2009). These results are similar to those previously reported by (Uddin *et al.* 1988; Saha *et al.* 1989; Haque *et al.* 1993, 1994; Kohinoor *et al.* 1994; Rahman and Rahman 2003; Rahman *et al.* 2005 and Asadujjaman *et al.* 2013) during fry rearing experiments of various indigenous or exotic carp and barb species.

After completion of the experiment, the highest production was obtained in T_1 (6.83 Kg dec^{-1}) compared to those in T_2 (5.36 Kg dec^{-1}) and T_3 (4.14 Kg dec^{-1}) but there were no significant difference ($P>0.05$) between T_1 and T_2. It might be due to relatively lower numbers of fry stocked in T_1 than those of T_2 and T_3. Hence, the observed poor growth at higher stocking densities could be due to space limiting effect, stressful situation caused by supplementary feed, some variations in environmental parameters and less availability of natural food (Ahmed *et al.* 2017). This result was consistent with the findings of Monir and Rahman (2015) and Asadujjaman *et al*. (2013).

Overall, the highest growth, survival, and production of fingerlings were obtained in the seasonal nursing ponds on 1200 dec^{-1}stocking density compared to the ponds of 1600 dec^{-1}. Physico-chemical parameters of nursery pond water during the study period were within the acceptable range for nursery management, the growth of fingerlings to a greater extent was dependent on the quality and quantity of food available. In the present experiment, the amounts of feeds supplied in different ponds were based on the number of fingerlings stocked and the amount provided per fry was kept at the same level. Hence, the observed low growth at higher stocking densities could be due to less availability of natural food and any unfavorable changes in environmental parameters (Kohinoor *et al.* 1997). The present findings are very close agreement with those reported by (Kohinoor *et al.* 1994; Rahman and Rahman, 2003 and Rahman *et al.* 2004, 2005).

The current research is the first time attempt to demonstrate the effect of stocking density on the proximate composition of fish. However the result showed that there is only a significant variation was noted 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 and ash content was the highest in the highest stocking density. Higher protein (11.30%) and lipid content (5.39%) and lower ash content (4.27 %) was found in the treatment of T_1 where the stocking density was the lowest.

The economic analysis of the nursing systems was conducted to determine the economic return under low input management. The highest variable cost (BDT dec^{-1}) was recorded in T_3 (1356.01±10.24) followed by T_1 (1172.27±94.0) and T_2 (1232.95±13.85) while the significantly lower gross margin (BDT) was found in the T_3 (883.69±26.43) in those of T_2 (907.73±121.08) and T_1 (1147.06±68.40) due to the lowest individual weight of *A. testudineus* for higher stocking density. In contrast to the present study Monir and Rahman (2015) recorded the cost and benefit of Shing (*H. fossilis*) in nursing system and got the gross margin of BDT 1622.02 to 4569.54 dec^{-1}after 8 weeks nursing when the fish was fed with commercial feed. Rahman and Marimuthu (2010) observed the nursing of Thai koi (*A. testudineus*) gave a gross margin 4240.11to 5973.68 $dec^{-1}$from 8 weeks nursing. However, the lowest BCR was recorded in T_3 (1.65±0.03) followed by T_2 (1.80±0.15) and T_1 (2.04±0.07) which might be due to higher FCR and cost required for nursing of Thai koi in the treatment T_3 compared to T_1 and T_2 (Chakraborty *et al*. 2005; Khan *et al.* 2003 and Usmani *et al*. 2003).

CHAPTER 6

SUMMARY AND CONCLUSION

This study was conducted to observe the effect of stocking density on the growth performance of Thai koi in the nursing stage over a period of 30 days at Saidpur upazila in Nilphamari district. The water quality parameters and growth performance of Thai koi (*A. testudineus*) was investigated under different stocking density in the seasonal nursery pond. For this experimental purpose, three treatments were evaluated which each having three replications. The stocking densities were 1200, 1400, and 1600 dec^{-1} in T_1, T_2 and T_3 respectively. From the beginning of the experiment, all the experimental fish were fed with a commercial feed (Lily Fish Feed) which containing 35.53 % protein.

The water quality parameters such as temperature was ranged from 27.20 to 27.60 °C; transparency 26.98 to 33.50 cm, pH 6.34 to 8.70; dissolved oxygen 5.02 to 5.17 mgL^{-1}; total alkalinity 99.40 to 109.10mgL^{-1}; hardness 38.80 to 76.60 mgL^{-1} and ammonia 0.38 to 0.44mgL^{-1}.Significant differences were found between the treatments in case of transparency, dissolved oxygen, total alkalinity, hardness and ammonia. Observed all the water quality parameters of this current study were suitable for successful nursing of Thai koi.

For estimating the growth performance, sampling was done after 10 days and the weight (g) of Thai koi were recorded. At the end of the experiment, the average weight gain of Thai koi was 6.35±0.90, 4.27±0.23 and 3.50±0.24g respectively in T_1, T_2 and T_3. The differences in weight gain under three treatments were significant. The highest growth rate was found in T_1 in which stocking density was 1200 dec^{-1}. The SGR among the three treatments were 11.26±0.41, 10.10±0.29 and 9.66±0.25 in T_1, T_2 and T_3 respectively. The SGR among the three treatments were significant and the highest SGR was observed in the T_1 while the FCR was lowest in the T_1. The survival rates under the three treatments were 86.66%, 85.00% and 70.00% in T_1, T_2 and T_3 respectively. The best survival rate was obtained in T_1 in which the stocking density was the lowest. The highest production was found from T_1 while the lowest in T_3. The Benefit Cost Ratio (BCR) was evaluated at the end of the experiment. The best benefit was obtained from T_1 in which BCR was 2.04 and the other two treatments BCR was 1.80 in T_2 and 1.65 in T_3.

The results of the present study revealed that the individual fish growth was decreasing with increasing of stocking density. Based on the results of the experiment, farmers could be suggested to nursing Thai koi (*A. testudineus*) at lower stocking densities (1200 dec^{-1}) to get higher production in the short period of time in the seasonal nursery pond. The production of quality fingerling through application of this current finding might have important implications for the farmers to enhance the quality fingerling production in the seasonal nursery pond which ultimately will help to improve their economic and livelihood status.

In order to validate the findings of this research more field trials will be needed. As a follow up to this current research, future researches proposed are as follows:

- Effect of stocking density on the grow-out production of Thai koi in northern region of Bangladesh.
- Effect of stocking density on the health status of Thai koi in nursing and grow-out phage of northern region of Bangladesh.

REFERENCES

Ahmed GU, Hossain MM and Hossain MS. 2009. Histopathology of diseases of an air breathing teleost (*A. testudineus*) from fresh water fisheries of Bangladesh. International Journal of Sustainable Agricultural Technology. 5(4): 75-81.

Ahmed S, Hasan KR, Hossain M, Mahmud Y and Rahman MK. 2017. Adaptability of polyculture of stinging catfish (*H. fossilis*) in seasonal water bodies of greater northern region, Bangladesh. International Journal of Fisheries and Aquatic Studies. 5(1): 433-439.

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.

Alhassan EH, Abarike ED and Ayisi CL. 2012. Effects of stocking density on the growth and survival of *O. niloticus* cultured in hapas in a concrete tank. African Journal of Agricultural Research. 7(15): 2405-2411.

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

Alim MA. 2005. Developing a polyculture technique for farmer's consumption and cash crop Ph.D Dissertation. Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh. pp. 192.

Aminul IM. 1996. Qualities of water and soil in Aquaculture, Fish week compilation, Department of Fisheries Publication, Dhaka. pp. 96.

AOAC. 1997. Association of official Analytical Chemist. Official Methods of Analysis of AOAC International, 16^{th} edn, 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.

Asaduzzaman M, Salam MA, Wahab MA, Kunda M and Rahman MB. 2006. Effects of control of C/N ratio by low-cost carbohydrate addition on water quality and pond ecology in freshwater prawn (*Macrobrachium rosenbergii)* post-larvae nursing system. Bangladesh Journal of Fisheries Research. 10(2): 121-130.

Backie 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.

Barton BA and Iwama GK. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Reviews of Fish Diseases. 10: 3-26.

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

Begum F. 1998. Effectiveness of urea along with a constant quality of poultry manure on plankton production in fish ponds. M.S Thesis, Department of Aquaculture, Bangladesh Agricultural University, Mymensingh. pp. 15-21.

Begum M, Mamun AA, Pal HK, Islam MA and Alam MJ. 2008. Effects of stocking density on growth and survival of *M. gulio* in nursery ponds of Bangladesh. Bangladesh Journal of Fisheries Research. 12(2): 179-186.

Bhattacharya S. 2013. A study on compensatory growth performance of Thai koi (*A. testudineus*) fed on different levels of satiation and restricted ration. M.S Thesis, Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh. pp. 19-29.

Beveridge MCM and Little DC. 2002. The history of aquaculture in traditional societies. Ecological aquaculture. The evolution of the Blue Revolution. pp. 3-29.

BFRI. 1997. Research progress, Bangladesh Fisheries Research Institute. Mymensingh, Bangladesh. pp.113.

Bijukumar. 2000. An exotic fishes and freshwater fish diversity. Zoos' Print Journal. 15(11): 363-367.

Boyd CE. 1992. Water quality in ponds for aquaculture. Alabama Agricultural Experiment station, Aubum University, Aubum. pp. 477.

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

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

Chakraborty BK and Mirza MJA. 2007. Effect of stocking density on survival and growth of endangered bata (*L. bata,* Hamilton–Buchanan) in nursery ponds. Aquaculture. 265: 156-162.

Chakraborty SB and Banerjee S. 2010. Effect of stocking density on monosex Nile tilapia growth during pond culture in India. World Academy of Science, Engineering and Technology. 44: 1521-1525.

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.

Chiu YN, Sumagaysay NS and Sastrillo MGS. 1987. Effect of feeding frequency and feeding rate on the growth and feed efficiency of milkfish (*Chanos chanos*) juvelines. Asian Fisheries Science. 1: 27-31.

Chowdhury MR. 2009. Population challenge facing Bangladesh. Long Island University, CW Post Campus, New York. http://bangladeshwatchdog.blogspot.com/2009/04/population-challenge-facing-bangladesh.html.

Das I and Ray AK. 1989. Growth performance of Indian major carps (*L. rohita*) on duck weed incorporated pelleted feed, a preliminary study. Journal of Inland Fisheries. 21: 1-6.

Daudpota AM, Kalhoro IB, Shah SA, Kalhoro H and Abbas G. 2014. Effect of stocking densities on growth, production and survival rate of red tilapia in hapa at fish hatchery Chilya Thatta, Sindh, Pakistan. Journal of Fisheries. 2(3):180-186.

De Silvia SS and Davy FB. 1992. Fish nutrition research for semi-intensive culture system in Asia. Asian Fisheries Science. 5: 129-144.

DoF. 2016. National fish week, 2016 compendium (In Bengali), Department of Fisheries, Ministry of Fisheries and Livestock, Government of the people’s Republic of Bangladesh. pp. 13-132.

DoF. 2013. National fish week, 2013 compendium (In Bengali), Department of Fisheries, Ministry of Fisheries and Livestock, Government of the people's Republic of Bangladesh. pp. 1-144.

DoF. 1996. Matshapakkha Sankalan (Bengali) Department of Fisheries, Ministry of Fisheries and Livestock, Government of the people's Republic of Bangladesh. pp. 81.

Doolgindachbaporn S, Jaruratjamom P and Khongsai S. 2003. Culturing of climbing perch (*A. testudineus*) at varying stocking densities. Department of Fisheries, Faculty of Agriculture, Khon Kaen province, Thailand. Journal of Environment and Ecology. 20(2): 55-60.

Farid SM, Miah MI, Habib MAB, Hasan MM and Ahammad AKS. 2009. Nursing and rearing of *M. aculeatus* at different stocking densities in pond culture system. Journal of Agroforestry and Environment. 3(1): 101-104.

FAO. 2014. The state of world fisheries and aquaculture: opportunities and challenges. Food and Agriculture organization of the United Nations, Rome. pp. 1-243

Ferdous Z, Masum MA and Ali MM. 2014. Influence of stocking density on growth performance and survival of monosex tilapia (*O. niloticus*) fry. International Journal of Research in Fisheries and Aquaculture. 4(2): 99-103.

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.

Fry FEL. 1971. The effect of environmental factors on the physiology of fish. Academic press, New York. 6: 1-98.

FRSS. 2015. Fisheries resources survey system (FRSS), fisheries statistical report of Bangladesh, Department of Fisheries, Bangladesh. pp. 1-5.

Ghosh SK, Mandal BK and Borthakur DN. 1984. Effects of feeding rates on production of common carp and water quality in paddy-cum-fish culture. Aquaculture. 40: 97-101.

Gokcek CK and Akyurt I. 2007. The effect of stocking density on yield, growth and feed efficiency of Himri Barbel (*B. luteus*) nursed in Cages of Turkey. The Israeli Journal of Aquaculture – Bamidgeh. 59(2): 99-103.

Habib KA, Newaz AW, Badhon MK, Naser MN and Shahabuddin AM. 2015. Effects of stocking density on growth and production performance of cage reared Climbing Perch (*A. testudineus*) of high yielding Vietnamese stock. World Journal of Agricultural Sciences. 11(1): 19-28.

Hakim A, Hussein NF, Bakeer MN and Soltan, MA. 2001. Effect of protein level and stocking density on growth performance of Nile tilapia (*Oreochromis niloticus*) cultured in tanks. Egyptian Journal of Nutrition and Feeds, 4: 763-780.

Haque MR. 2000. An evaluation on the potential of periphyton based culture of Mohashol (*T. putitora*, Hamilton). M.S. dissertation. Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh. pp. 42.

Haque MZ, Rahman MA and Hossain MM. 1993. Studies on the effect of stocking densities on the growth and survival of mrigal (*C. mrigala*) fry in rearing ponds. Bangladesh Journal of Zoology. 21(1): 51-58.

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

Hasan MA. 1998. Development of carp polyculture techniques with small indigenous fish species Mola (*A. mola*), Chela (*Chela cachius*), punti (*Puntius sophore*). M.S Thesis, Department of Fisheries Management. Bangladesh Agricultural University, Mymensingh. pp. 71.

Hasan SJ, Mian S, Rashid AHA and Rahmatullah SM. 2010. Effects of stocking density on growth and production of gift (*O. niloticus*). Bangladesh Journal of Fisheries Research. 14(1-2): 45-53.

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

Islam MS. 2002. Evaluation of supplementary feeds for semi-intensive pond culture of mahseer (*T. putitora*, Hamilton). Aquaculture. 212: 263-276.

Islam MS, Dewan S, Hussain MG, Hossain MA and Mazid MA. 2002. Feed utilization and wastage in semi-intensive pond culture of mahseer (*T. putitora,* Hamilton). Bangladesh Journal of Fisheries Research. 6: 1-9.

Jhingran VG. 1991. Fish and Fishes of India (3rd ed.). Hindustan Publication of Corporation, New Delhi. pp. 727.

Jiwyam W and Nithikulworawong N. 2014. Stocking density-dependent growth and survival of Asian red-tailed catfish (*H. wyckioides*) fries early nursing in cages. International Aquatic Research. 6: 245–250.

Khan MN, Islam AKMS and Hussain MG. 2003. Marginal analysis of culture of stinging catfish (*H. fossilis*) effect of different stocking densities in earthen ponds. Pakistan Journal of Biological Science. 34: 1365-2109.

Kohinoor AHM, Haque MZ, Hussain MG and Gupta MV. 1994. Growth and survival rate of Thai punti (*Puntius gonionotus*, Bleeker) spawn in nursery ponds at different stocking densities. Journal of the Asiatic Society of Bangladesh. 20: 65-72.

Kohinoor AHM, Hossain MA and Hussain MG. 1997. Semi-intensive culture and production cost of pabda (*Ompok pabda*) with rajpunti (*P. gonionotus*) and mirror carp (*Cyprinus carpio* var. *specularis*) in mini ponds. Bangladesh Journal of Zoology. 25: 129-133.

Kohinoor AHM, Islam AKMS, Jahan DA, Zaher M and Hussaian MG. 2007. Monoculture of climbing perch, Thai koi (*A. testudineus)* under different stocking densities at on-farm. Bangladesh Journal of Fisheries Research. 11(2): 173-180.

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, 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.

Le Cren ED. 1965. Some factors regulating the size of population of Freshwater Mitteilungen. International Vereinigung fur Theoretische and Angewandte Limnology. 13: 88-105.

Masud MA, Zoha M, Begum NN, Ali MZ and Nahar F. 1996. Formulation of cost effective feeds from locally available ingredients in carp polyculture system for increase production. Aquaculture. 151: 71-78.

Mensah ETD, Attipoe FK and Johnson MA. 2013. Effect of different stocking densities on growth performance and profitability of *O. niloticus* fry reared in hapa-in-pond system. International Journal of Fisheries Aquaculture. 5: 204-209.

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.

Mustafa MG, Alam MJ and Islam MM. 2010. The effects of some artificial diets on the feed utilization and growth of the fry of climbing perch (*A. testudineus*). Rep. Opinion. 2: 3-28.

NFP. 1998. National Fisheries policy, Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh.http://www.fisheries.gov.bd/site/view/policies/Policy.

Nirod DB. 1997. Effect of stocking density on the growth and production of Mola (*A. mola*). M.S Thesis, Department of Fisheries Management. Bangladesh Agricultural University, Mymensingh. pp. 75.

Pankhurst NW and Kraak GV. 1997. Effects of stress on reproduction and growth of fish, in fish stress and health in aquaculture. Cambridge University Press, Cambridge. pp. 73-93.

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.

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.

Rahman MA, Arshad A, Amin SMN and Shamsudin MN. 2012. Growth and survival of fingerlings of a Threatened Snakehead (*C. striatus*) in earthen nursery ponds of Bangladesh. Asian Journal of Animal and Veterinary Advances. pp. 1-11.

Rahman MA and Marimuthu K. 2010. Effect of different stocking density on growth, survival, and production of endangered native fish climbing perch (*A. testudineus*, Bloch) fingerlings in nursery ponds of Bangladesh. Advances in Environmental Biology. 4(2): 178-186.

Rahman MA, Mazid MA, Rahman MR, Khan MN, Hossain MA and Hussain MG. 2005. Effect of stocking density on survival and growth of critically endangered mahseer (*T. putitora*, Hamilton), in nursery ponds. Aquaculture. 249: 275–284.

Rahman MA and Rahman MR. 2003. Studies on the growth and survival of sharpunti (*P. sarana,* Hamilton) spawn at different stocking densities in single stage nursing. Progress Agriculture. 14(1-2): 109-116.

Rahman MA, Zaher M and Azimuddin KM. 2009. Development of fingerling production techniques in nursery ponds for the critically endangered reba carp (*Cirrhinus ariza*). Journal of Fisheries Aquatic Science. 9: 165-172.

Rahman MN. 2000. Effects of addition of calibaus (*L. calbasu)* in the periphyton-based aquaculture systems of rohu and catla. M.S. Thesis, Department of Fisheries Management, Bangladesh Agricultural University, Mymensingh. pp. 82.

Rahman MR, Rahman MA and Hussain MG. 2004. Effects of stocking densities on growth, survival and production of calbasu (*L. calbasu,* Hamilton) in secondary nursing. The Bangladesh Veterinarian. 21(1): 58-65.

Rahman S and Monir MS. 2013. Effect of stocking density on survival, growth and production of Thai koi (*A. testudineus*) fingerlings under nursery pond 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 SMS. 2005. Effect of stocking density of gift strain of Nile tilapia (*O. niloticus*) and freshwater prawn (*Macrobrachium rosenbergii*) in periphyton based production system. M.Sc. dissertation, Dept. of Fisheries Management, Bangladesh Agricultural University, Mymensingh. pp. 84.

Ramadan A and Seif A. 2006. Effects of stocking density and protein level in diet on growth performance, survival rate and feed efficiency of Nile Tilapia *(Oreochromis niloticus*) fry monosex during the nursery period. Egyptian Journal of Aquatic Biology and Fisheries, 10(3): 69-84.

Reddy SR and Katro S. 1979. Growth rate and conversion efficiency of air breathing catfish (*H. fossilis)* in relation to ration size. Aquaculture. 18: 35-50.

Ronald N, Gladys B and Gasper E. 2014. The effects of stocking density on the growth and survival of Nile tilapia (*O. niloticus*) fry at Son fish farm, Uganda. Journal of Aquaculture Research and Development. 5: 222.

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.

Saha KC. 1971. Fisheries of West Bengal, West Bengal Government Press, Alipore, West Bengal, India. pp. 120.

Saha SB, Gupta MV, Hussain MG, Shah MS and Rahman MM. 1989. Effect of different fertilizers on the growth and survival of silver carp (*H. molitrix*) spawn in nursery ponds. Bangladesh Journal of Zoology. 17: 57-67.

Sarker MN. 2000. Effects of periphyton on monoculture of gift tilapia, M.S. Thesis, Department of Fisheries Management. Bangladesh Agricultural University, Mymensingh. pp. 88.

Shariful ME, Ali H, Alam MS, Hossain MM and Rahman MO. 2009. Depth wise abundance of benthic fauna in a selected pond of Mymensingh district. Bangladesh Journal of Agriculture. 2(1): 29-34.

Shelton WL and Rothbard S. 2006. Exotic species in global aquaculture - a review. The Israeli Journal of Aquaculture – Bamidgeh. 58(1): 3-28.

Uddin MM. 2002. Effect of addition of small fish on pond ecology and production in polyculture M.S Thesis, Department of Fisheries Management. Bangladesh Agricultural University, Mymensingh. pp. 81.

Uddin MS, Gupta MV and Barua G. 1988. Effect of fertilizers on the growth and survival of rohu (*L. rohita*) spawn in nursery ponds. Bangladesh Journal of Fisheries. 11: 83-88.

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.

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.

APPENDICES

Appendix A: Water Quality Analysis

A.1 Analysis of alkalinity

Determination of Phenolphthalein Alkalinity

- Removed the cap from the small plastic vessel. Rinsed the plastic vessel with water sample, fill to the 5 ml mark and replace the cap.
- Added 1 drop of phenolphthalein indicator through the port cap 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 the next step.
- Taken the titration series and pushed plunger completely into HI3811-0 solution and plunger seal is on the 0 ml mark of the syringe.
- Placed 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 in the plastic vessel turns colorless.
- Read off the millimeters of titration solution from the syringe scale, and multiply by 300 to obtain mgL^{-1} (ppm) $CaCO_3$.

Determination of Total Alkalinity

- Removed the cap from the plastic vessel. Rinsed 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 proceed next step.

- Taken 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.
- Placed 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 mgL^{-1} (ppm) $CaCO_3$.

A.2 Analysis of hardness

- Removed the cap from the small plastic beaker. Rinsed the plastic beaker with the water sample, fill to the 5 ml mark and replace the cap.
- Added 5 drops of Hardness Buffer through the port cap and mix carefully swirling the beaker in tight circles.
- Added 1 drop of calmagite indicator through the port cap and mix as described above. The solution becomes a red-violet color.
- Taken 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.
- Placed 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 mgL^{-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 to the sample used, weighed out these petridish by using an electronic balance and was recorded.
- Then the sample ingredients were weighted in the clean weighted 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 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 + $CuSO_4$ 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

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

% Crude protein in sample = % Nitrogen × 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:

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

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 porcelin crucible with the remaining ash is recorded.
- % ash is calculate

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