Effects of salinity changes on growth performance and survival of Climbing Perch, *Anabas testudineus* (Bloch, 1795)

A thesis submitted in partial fulfillment of the requirements for the Degree of Master of Science in Fisheries.

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Certificate

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Dedicated To
My Beloved Parents

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ABSTRACT

An experiment was carried out at aquatic laboratory, Department of Fisheries, University of Dhaka, Bangladesh in order to find out the salinity tolerance capability and growth performance of climbing perch (*Anabas testudineus,* Bloch 1795)". There were four hundred (400) of *A. testudineus* fingerlings collected from a reputable fish hatchery in Mymensingh of Bangladesh. Their length and weight ranged between 1.2 to 5.5 cm and 1.07 to 7.8 g respectively. *A. testudineus* commonly cultured fish in Bangladesh was reared in laboratory condition at different salinities of 0, 3, 6, 9, 12, 15, 18 and 21‰ for 60 days. Hundred percent survivals were detected at 0, 3, 6 and 9‰ salinity while 100% mortality was recorded at 18 and 21‰ salinity. Variable behavioral responses to threat and feeding were observed among the fish in different treatments. Variable levels in different growth parameters were found during the study period. Lowest feed conversion ratio was found in control group while the highest was detected at 15‰ salinity. On the other hand, decreasing trend of specific growth rate and average growth rate was observed in *A. testudineus* fingerlings from 0 to 15‰ salinity. Significantly higher specific growth rate and average growth rate was detected in *A. testudineus* fingerlings reared at (0-6) ‰ salinity ($p<0.05$). The specific growth rate was 1.16 ± 0.05^{a}, 0.99 ± 0.00^{b} and 0.96 ± 0.01^{b} at 0, 3 and 6‰ salinity respectively. The average growth rate was 0.14 ± 0.01^{a}, 0.12 ± 0.02^{ab} and 0.12 ± 0.01^{ab} at 0, 3 and 6‰ salinity respectively. Thus the present study suggests that *A. testudineus* fingerlings can be reared in coastal water with salinity up to 6‰ as similar growth rate of freshwater.

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List of Symbols and Abbreviations

Symbols	Details
°C	Degree celcious
G	Gram
mg	Milligram
cm	Centimeter
mL	Milliliter
cm	Centimeter
%	Percentage
‰	Salinity
ANOVA	Analysis of variance
SRDI	Soil Resource Development Institute
DOF	Department of Fisheries
FCR	Feed conversion ratio
SGR	Specific growth rate
AGR	Average growth rate
SPSS	Statistical Package for the Social Sciences

Chapter 01
Introduction

CHAPTER 01
INTRODUCTION

1.1 General Background

Climbing Perch (*Anabas testudineus*) commonly known as koi fish in Bangladesh is highly delicious and having more demand to the consumer. As a freshwater fish species *Anabas testudineus* is included in Perciformes order and undergoes Anabantidae family. Freshwater fish is much more important for national economy and as a major source of protein, essential minerals, vitamins, and unsaturated fats . Bangladesh is an agro-based riverine country enriched with vast fisheries resources. The total area of inland water in general estimated about 46,99,345 hectares. Water area of inland fisheries including rivers, beels, Kaptai lake, flood plains and polder/enclosure as open water body (capture based) comprise about 39,25,290 hectares, ponds and ditches, oxbow lake and coastal shrimps farm as closed water body (culture based) comprise about 7,74,055 hectares. The republic has a 710 km long coastline and approximately 1,66,000 square kilometers of seawater area. The nation's exclusive economic zone contains up to 200 nautical miles from the coastline. Thus the nation's total area of water having fish production potential is of very great importance. Fisheries play a vital role in our national economy and contribute 4.39% to the GDP, 22.76% to the agricultural products and 2.46% to the export earnings. In 2011-2012 Bangladesh earned 4,703.95 crore taka by exporting 92,479 metric tons of fish and fishery products to foreign countries. About 10 million people are directly and indirectly engaged in subsistence fishing and activities related to fisheries sector (DOF, 2011-2012).

Fish is the major protein sources in the diet of the Bengali people. Fish contributes about 60% of the available protein in the diet and the rest 40% protein comes from livestock and poultry (DOF, 2011-2012). It indicates the importance of fish in contributing to the level of nutrition of the people of Bangladesh. In spite of having large fisheries resources, Bangladesh is facing an acute malnutrition problem due to the shortage of animal protein supply in our diet. The present per capita fish consumption is about 41.89 g/day whereas 56 g/day is the required amount (DOF, 2011-2012). This is due to rapid human population growth and decline of catch from inland open water area. There is a little prospect for additional yield from open water capture fisheries. Only the culture fisheries, seems to be dependable means of the achieving increased yield. In order to meet the need for vast increase in animal protein supplies, animal breeders introduced new high yielding varieties of live stocks, aqua culturist introducing new methods of fin fish, shell fish and crustacean culture to enable animal protein production to keep with the increase in population.

Growth performance of fish largely depends on feed consumption and its assimilation and conversion into body tissues (Brett and Groves, 1979; Burel *et al.*, 1996). However, growth and food intake are controlled by internal factors involving the central nervous system, endocrine system, and peripheral nervous system, and also by environmental

factors such as temperature, photoperiod, salinity, oxygen level, ammonia and P^H. A number of experiments showed that salinity is one of the most significant environmental parameters influencing survival, growth and distribution of fish both in freshwater and marine habitat (Arunachalam and Reddy, 1979; Tandler *et al.*, 1995; Peterson *et al.*, 1999; Imsland *et al.*, 2001). Water salinity can affect survival of fish by diminishing feeding rate (Dendrinos and Thorpe, 1985; Fielder and Bardsley, 1999), and by modifying the energetic cost for osmotic and ionic regulation (Boeuf and Payan, 2001).

Arunachalam and Reddy (1979); Lilongwe *et al*., 1996; Water salinity affects the growth of freshwater species. Alava (1998); Imsland *et al*., (2001); Salinity can modify the feed conversion efficiency. Brett and Groves, (1979); Burel *et al*., (1996) Growth of fish depends on consumption of feed and its assimilation and conversion into body tissues. Boeuf and Payan (2001); Attributed the growth enhancement at iso-osmotic salinities in stenohaline and euryhaline fish species to several causes, i.e., reduction of energetic costs for ionic and osmotic regulation, increased food intake, food conversion and stimulation of hormones supporting growth. Dendrinos, and Thorpe (1985); Imsland *et al*., (2001); Salinity can modify the total food intake. Foss *et al*. (2001) ; working on spotted wolfish, *Anarhichas minor*, did not report any effect of salinity on feeding rate, feed conversion or protein efficiency ratio, although indications of enhanced growth at iso-osmotic salinity were discussed. Francois *et al*., (2004); Ferraris *et al*., (1986); Reports of a slower intestinal transit of nutrients at the intermediate salinities in the Atlantic wolfish, *Anarhichas lupus*. Gaumet *et al*., (1995); Woo and Kelly (1995), Dutil *et al.* (1997); The energetic cost for homeostasis (i.e. ionic and osmotic regulation) is reduced in iso-osmotic environments and that saved energy translates into growth. Handeland *et al*. (2000), Boeuf and Payan (2001); Salinity can modify a number of aspects related to the balance of hormones involved in metabolism. Lambert *et al*. (1994); Gaumet *et al*. (1995); Imsland *et al.*(2001); A prolonged digestion time for nutrient absorption that could translate into better digestibility and increased energy available for somatic growth. Marine species such as Atlantic cod, *Gadus morhua*, and turbot, *Scophthalmus maximus*, have also been shown to exhibit increased growth rates (cod), feed conversion ratios (cod), and food intake (turbot) when cultured at intermediate salinities of 12-19%.

Nordlie *et al*. (1991); Woo and Kelly (1995); Salinity can modify a number of aspects related to growth including metabolic rate. Rubia *et al*. (2005); Growth and food intake are controlled by internal factors involving the central nervous system, endocrine system, and neuroendocrine system, and also by environmental factors such as temperature, photoperiod, salinity, oxygen level, ammonia and pH. Salinity, like other environmental factors specific to aquatic habitats, has prompted many studies on its influence on fish growth. Resley *et al*. (2006); cobia, *Rachycentron canadum*, reared at a salinity of 5‰ grew as well or better than conspecifics reared at 15 and 30%. Saunders and Henderson (1970); Buckel and Steinberg *et al*. (1995), there appear to be few species whose growth is not affected by salinity. Shaw *et al*., (1975); a range of salinity. i.e., 0.1, 10 and 20‰

did not influence growth of Atlantic salmon, *Salmo salar.* Tandler *et al*. (1995); Peterson *et al*. (1999); Imsland *et al.* (2001); water salinity affects the growth of marine water fish species.

Salinity like any other environmental factors specific to aquatic habitats has prompted many studies on its influence on fish growth (Rubia *et al.*, 2005). It has been proposed that the energetic cost for homeostasis (i.e. ionic and osmotic regulation) is reduced in iso-osmotic environments and that saved energy translates into growth (Boeuf and Payan, 2001; Imsland *et al.*, 2001). Salinity can potentially act as a stressor under both natural and aquaculture conditions (Varsamos *et al.*, 2004). Growth and survival to changes in salinity may provide a bio-energetic basis to evaluate performance of *A. testudineus* under culture conditions. This is also relevant for the culture of salinity-tolerant aquaculture candidates which could be reared at different salinities.

1.2 Rationale

Bangladesh is extremely vulnerable to climate change because of its geophysical settings. It is a low-laying flat country with big inland water bodies, including some of the biggest rivers in the world– Brahmaputra, Ganges and Meghna. Additionally Bangladesh is intensified by tropical cyclones and monsoon rainfalls. Therefore, disastrous floods took and continue to take place here. Each time during the heavy river floods in 1992 and 1998 more than half of the national territory was flooded. In 1970 and 1991, tropical cyclones forced storm surges killing some hundred thousand people. The storm of 1970 forced a storm surge of 9 metres. Houses, crops and hundreds of thousands of livestock were swept away. Salinity intrusion to the coastal belt of the country is the regular outcome due to sea level rise for climate change. Altinok and Grizzle (2004), Hogendoorn *et al. (*1981); An increase in energy expenditure on excretion could influence energy balance and therefore growth efficiency, Metabolism, which includes all processes where transfer of energy is involved, can be quantified on the basis of the energy expenditure, the energetic cost of life. Dutil *et al.* (1992) and Audet *et al.* (1993); Osmo-ionic adjustments of juvenile *L. lithognathus* could be evaluated after transfer of fish to different salinity treatments by measuring the physiological variables such as plasma osmolality, sodium (Na+), chloride (Cl-), potassium (K+) and water content of skeletal muscle at regular intervals following the methods .

Deacon (1997); Bacela, (2002); In commercial aquaculture knowledge and an understanding of the effects of the environmental factors on the physiology of a candidate aquaculture species are prerequisites for successful aquaculture. Kaseloo *et al*. (1992), Cooke *et al*. (2000); one of the best indicators of activity levels are integrated electromyograms (EMG*i'*s) from axial musculature. EMG*i* activity is proportional to locomotory activity and can also be correlated to oxygen consumption usingrespirometry. Lucas *et al*. (1993); the development of telemetric techniques has allowed activity of fish to be studied *in situ.* Morgan and Iwama (1991); Boeuf and Payan (2001), Bayne (1975); Environmental salinity is one of the most important factors in fish physiology particularly in estuarine-dependent and marine aquaculture candidates.

Physiological rates can be measured across a salinity gradient and converted to energetic equivalents to determine the effect of salinity on certain components of the energy budget of fish. Morgan and Iwama (1991); Secor *et al*. (2000); the relationship between growth and salinity is complex, as it is species-specific, and may differ between populations or life-history stages. Nordlie and Haney (1998) reviewed results from studies on twelve euryhaline fish species that utilise habitats from fresh water to marine waters and found that different species with similar habitat utilisation showed different combinations of physiological responses to ranges of ambient salinity. Nordlie *et al*. (1991); Woo and Kelly, 1995; Frick and Wright, 2002; Energy expenditure of fish can be estimated as a function of salinity by measuring metabolic rate and the release of nitrogenous compounds. Nordlie (1985); In a review of the osmotic regulatory ability of euryhaline fishes, concluded that the affinity of a species for fresh water, brackish and marine habitats is not directly related to their ability to maintain their plasma osmotic concentration.

Rankin and Bolis (1984), Jones and Randall, 1978; Other physiological processes unrelated or only indirectly related to the energetics of osmoregulation such as tissue permeability to water and ions, gill ventilation, perfusion, functional surface area and permeability play an important role in salinity adaptation. Sparks *et al*. (2003); Acclimation to changes in environmental salinity involves several hormonal and osmoregulatory adjustments in order to re-establish ionic homeostasis. Saillant *et al*. (2003); Salinity may influence physiological mechanisms involved in growth such as the secretion of growth hormone or prolactin. Swanson (1998); while activity of fish may have affected osmoregulatory costs, salinity may also have affected activity in 71 ways that compensate for elevated maintenance costs and/or control or minimise activity-related costs of osmoregulation. This has been reported in milkfish, *Chanos chanos*,). Woo and Wu,(1982); Gaumet *et al*., (1995); Claireaux and Lagardere, (1999); Resley *et al*., (2006); Despite many reports about the influence of salinity on aspects of growth performance of fish accounts of the effects of salinity on oxygen consumption and growth of estuarine-dependent and marine teleosts in southern Africa are lacking. Some information on the ecology, osmoregulation and reproductive biology of the euryhaline marine white steenbras, *Lithognathus lithognathus*, is available, especially 3concerning juveniles in estuaries. *Lithognathus lithognathus* is endemic to southern Africa, occurring between the mouth of the Orange River and Natal. Ahmed *et al*. (1991) worked on the effects of aeration on growth and survival of *Clarias gariepinus* larvae under culture. *Clarias gariepinus* larvae of 8 days old were reared in six fiber glass tanks under continuous aeration facilities and without aeration .The level of dissolve oxygen concentration was 4.5-6.0 mg/L in the aerated tanks and 3-7 mg/L in the non aerated tanks. An increased growth rate and survival percentage of catfish larvae were recorded from the tank provided with aeration when compared with the non aerated ones.

Ahmed *et al*. (1997) worked on the culture feasibility of African catfish (*Clarias gariepinus,* Linn.) fry in glass tank and synthetic hapa system using supplemental diets.

Effects of different feeds on growth and survival of African cat fish fry (10 days old) were determined in glass tanks for a period of 42 days. Two tanks and two ponds were taken for the experiment. One tank was named as tank tubifex and another tank was named as tank sabinco. One pond as pond tubifex and the other pond as pond sabinco. Live tubifex (protein 64.48%) was supplied to tank tubifex and pond tubifex and sabinco was supplied to the other two treatments. Growth of cat fish fry in tubifex pond in terms of both length and weight were significantly higher than those of the other treatments. In terms of growth performance and survival rate tubifex pond showed better result. Akand *et al*. (1989) reported on the dietary protein requirement of stinging catfish, *Heteropneustes fossilis* (Bloch) while they worked on the effect of dietary protein on the growth, feed conversion and body composition of shingi fish in aquarium.

Alam and Mollah (1998) investigated on the effect of stocking density on the growth and survival of magur *Clarias batrachus* (Linn).The experiment was made for period of 4 months, starting from 1st September to31st December 1987 in the fisheries Research institute, Mymensingh, Bangladesh. The study was carried out according to 3 x 3 randomized block design. Three stocking density, viz., 2,500/ha, 5,000/ha and 10,000/ha were employed. In the experiment it was revealed that decreased stocking density favors increased growth rate and vice versa. Growth rate retarded gradually in respect of length and weight. On the other hand, the stocking density had a significant influence upon length and weight ($p<0.01$) but survival percentage remain same. Alam and Mollah (1998) conducted an experiment for a period of 20 days to develop artificial dry feed for nursing of *Clarias batrachus* larvae. They formulated four feeds with fish meal and backsets as mean sources of live tubifex species in relation to growth and survival of larvae. Larvae fed on live feed exhibited superior growth response. Boonyaratplin (1997) while working with nutrient requirements of marine feed fish cultured in Southeast Asia reported that the optimal dietary lipid concentration for juvenile Asian Sea bass between 6%and l8%. Catacutan and Coloso (1995) while working with effect of dietary protein to energy rations on growth, survival and body composition of juvenile Asian sea bass *Lates calcarifer* reported that the optimal crude protein (CP) specification of dry pelleted diets for juvenile Asian sea bass between 40%and 55%. Cruz and Laudenccia (1978) studied the protein requirements of Nile tilapia (*Oreochromis niloticus*) fingerlings and concluded that 20-30% Crude protein was required in the ration for optimum gowth and production. Das *et al* (1992) reported the growth of *Clarias batrachus* had an inverse relationship with the stocking density. They recommended that the lower stocking density showed the highest growth. Fracalossi and Lovell (1995) while working with growth of Channel catfish (*Ictalurus punctatus*) feed various lipid sources at two water temperatures reported that at 28°C Young catfish fed a diet with menhaden oil or a combination of menhaden oil, beef tallow, and corn oil had significantly greater weight gains than fish fed only beef tallow, corn oil, or linseed oil. At l7°C fish fed diets with menhaden oil or the mixture gained significantly more weight than those fed beef tallow.

Gheyas (1998) carried out an experiment on rearing of larvae of *H. fossilis* with three feeds viz. Tubificid woffns, a formulated feed and a commercial nursery feed marketed

under the trade name of SABINCO. She reported that Tubificid worms gave the best result in the term of growths and survival rate. Tubificid worms showed significantly higher survival rate, but no significant difference was observed between the formulated feed and commercial nursery feed. Hasan *et al.* (1982) studied on the growth of Nile tilapia in terms of increase in length and weight under four different dietary conditions for a period of three months in different floating ponds. They reported that variation in gain length were not statistically significant. Supplemental feed containing rice bran and fishmeal was found to give the highest growth rate. Hasan *et al*. (1982) found that 30 fish / m3 was the best stocking density of Nile tilapia, *O. nilotica* in floating ponds. Islam *et al* (1996) carried out an experiment to find out suitable feed and density to rear shingi fiy in laboratory condition with three different types of feeds and they observed that growth rate and survival rate of shingi fry were significantly high in life *Tubifex*.

Jhingran, (1982) pointed out that *H. fossilis* fingerlings stocked at 2, 50,000 / ha and fed with rice dust. Cut weed-fish-mixed with cattle dung yielded a production equivalent to 4.4 t/ha in 4 months at 80% survival. Khan and Sarkar (2004) while working with improvement in pangas (*Pangosius hypophthalmns*) farming by using silver carp (*Hypophthalmichthys molitrix*) to reduce the effect of eutrophication reported that a 50% replacement of pangas by silver carp showed higher growth rate, net income, better water quality (dissolved oxygen, nitrate-nitrogen and phosphate-phosphorus levels) and controlled blue-green algal bloom. Kibria *et al*. (1997) made an experiment on preliminary rearing trials of an Australian native fish. Silver perch with reference to growth and production of solid waste in Aquaculture in aquaria and fasted and commercial diets, to the diet l, 2 and 3 with protein content 53, 45 and 36% respectively .The initial average weight of those fishes were 1.54, l.14 and I '44 gm and after four weeks average weight were 1.98, I .6 and I .83 g.

The water salinity affects the fish directly or indirectly through generating changes in the ecological factors. A change in salinity effects the survival and growth of fish (Muir and Roberts, 1995). Due to these high levels of salinity, the traditionally cultured fish species fall under stress and could not performed well. So they have shown stunted growth and low production. Since salinity tolerance of fish is often positively correlated to growth capacity (Lemarie *et al.*, 2004), and metabolism is a major factor influencing the proportion of energy intake allocated to growth (Sun *et al.*, 2006). For the Aqua culturists, it is important to determine the optimum level of salinity for the cultured fishes.

Although the effect of salinity on aquatic organisms are still undervalued. Most of the country takes initiative to adopt this adverse condition by doing several research works on salinity and its effect on aquatic organism but unfortunately Bangladesh is far away from this track. So immediate research should be needed to resolve the production loss and as well as extinction of fish species due to salinity increase.

1.3 Research gap

Climate change is accelerating rapidly. Already, many countries, ecosystems and people are suffering from its impacts. Climate change has affected our weather patterns and disrupted our variability and trends in climate. In 1991, the Intergovernmental Panel on Climate Change IPCC raised the alarm globally by presenting scientific findings on evidence of global warming, emission increase and climate change impacts. This resulted in a worldwide recognition that some serious actions are necessary to save our planet. In 1992 the UN Climate Convention led to the establishment of an inter-governmental process to identify and implement necessary response measures to curb climate change and address its negative impacts.

Due to climate change all of the parameters specially related to the aquatic eco-system are changing day by day. Salinity difference is one of the most important parameter which is changing firstly for climate change. Fish farming in coastal region is becoming more risky due to salinity increase. Besides aquaculture industry has been flourished in a number of developing countries of tropical and sub-tropical zones (e.g. Bangladesh, Vietnam, Philippines, Thailand, China, India, and Mexico). Scientific and technical knowledge on effects of the environmental factors on the physiology of a target aquaculture species are prerequisites for successful aquaculture. A water salinity map for the period of 1967-1997 produced by Soil Resource Development Institute (SRDI, 1998) shows that the problems are already on the way. A comparative study between soil salinity map of (SRDI, 1998) for the period of 1967-1997 shows salinity intrusion in soil much higher than water salinity. A one meter sea level rise will expand the soil and water salinity area at a faster rate which drastically affects the culture practice of various species at the coastal belt.

It has already been mentioned that salinity is one of the most important factors in fish physiology (Boeuf and Payan, 2001) particularly in estuarine-dependent and marine aquaculture species. Physiological rates can be measured across a salinity gradient and converted to energetic equivalents to determine the effect of salinity on certain components of the energy budget of fish (Bayne, 1975). For example, energy expenditure of fish can be estimated as a function of salinity by measuring metabolic rate and the release of nitrogenous compounds. An increase in energy expenditure on excretion could influence energy balance and therefore growth efficiency (Altinok and Grizzle, 2004). Metabolism, which includes all processes where transfer of energy is involved, can be quantified on the basis of the energy expenditure, the energetic cost of life (Hogendoorn *et al.*, 1981). The scope for growth or the energy available for growth and reproduction can be estimated from these data, allowing for predictions of growth rates of fish (Guerin and Stickle, 1997).

At present in Bangladesh due to salinity change most of the marine water species are in danger. Aquaculture is a vital income generating sector for Bangladesh where more than six million people are directly involved. Bangladesh is going to be badly affected by global climate change and sea level rise. Millions of coastal inhabitants of Bangladesh

are going to lose their livelihoods and heritage. Particularly, agricultural and aqua-cultural activities will be severely affected by several physio-chemical factors especially by saline water intrusion. As an over populated and least developed country, it is all most impossible for the Bangladeshi government to relocate millions of people from the coastal region. Therefore, it is better to find the ways how to adjust with the adverse climatic conditions. So, for the enhanced economic growth, researches in several agricultural sectors like aquaculture, horticulture, paddy culture are important to develop new and sustainable technologies to adjust with the changed situation.

In Bangladesh very limited work has yet been conducted to understand the impact of salinity increase on survival and growth performance of fish. Understanding salinity tolerance based on measurement of metabolic rate and growth of *Anabas testudineus* fish fingerlings can help to evaluate the effects of salinity fluctuations on metabolism and growth performance of fish.

1.3 Objectives

The overall objective of this study was to investigate how salinity affects the growth of *Anabas testudineus* fingerlings in culture condition. The specific objectives were:

- Estimation of salinity tolerance limits of *A. testudineus* fingerlings as an initial step in order to determine the viability and potentiality of *A. testudineus* cultivation at different salinities.
- Determination of survival rate of *A. testudineus* fingerlings at different salinity levels.
- Evaluation of growth performances such as Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Average Growth Rate (AGR) of *A. testudineus* fingerlings at different salinity levels.
- Identification the feeding response and threat response at different salinities of *A.testudineus* fingerlings.

Chapter 02
Materials and Methods

CHAPTER 02
MATERIALS AND METHODS

2.1 Experimental fish

Anabas testudineus was selected for the purpose of experiment due to several reasons. There were four hundred (400) of *Anabas testudineus* fingerlings obtained from a reputable fish hatchery in Mymensingh area of Bangladesh. In the laboratory, biometric data on total length (TL) and body weight (BW) measurements were recorded for individuals. Their length ranged between 1.2 to 5.5 cm and weighed between 1.07 to 7.8 g.

The culture of this fish would prove a more profitable venture for the following reasons:

1) The *A. testudineus* having good consumer demand;

2) Comparatively moderate in market price;

3) It has more nutritious and of medicinal value.

4) It contains low fat and fewer intramuscular spines;

5) Its culture involves low risk and simple management;

6) It can be cultured in rejected, weed infested, silt-laden, foul water of shallow oxidation ponds,

8) They can tolerate high stocking density.

9) The fry withstands a wide range of salinity

10) It has high stress maintaining capability.

2.2 Description of the experimental site

The experiment was carried out at the aquatic laboratory of Department of Fisheries, University of Dhaka. The experiment consisted different series of activities like collection of feed, collection of experimental fish fingerlings, acclimatization of the fish in the laboratory condition, adopt them in to different salinities, applied feed in proper ration, water quality monitoring and as well as measuring the biological parameters required for growth performance, feed efficiency and survival.

2.3 Water Quality Monitoring

Temperatures, salinity, dissolved oxygen (DO) and P^H was monitored before and during each trial. Observing water parameters are the pre-requisite of any kinds of aquatic experiment (APHA, 1998). Salinity levels were measured using the salinometer, YSI, Japan, P^H levels were measured using the pH meter, ITOSENCE, Japan, DO levels were measured using the DO meter, ITOSENCE, Japan (Plate 1. a, 1.b, 1.c).

Tap water was used for the experiment and water parameters were measured for every 3 days in total 90 days rearing period. All water quality parameters were maintained within the normal range for fresh water fish culture (Alabaster and Lloyd, 1982). Water

parameters at the period of this study were P^H 8.1 to 9.6, DO 5.5 to 7.95 mg/l, and temperature 25.10 to 31.20^oC.

2.4 Acclimatization Tank

The fingerlings were acclimatized for a period of 72 hours prior to start of the experiment at the acclimatization tank. The rectangular acclimatization tank (Plate 1. d) was set-up in the Aquatic lab at the size of 80" ×60" ×40" with proper aeration system.

2.5 Brine Solution

The brine solution was prepared in the circular tank (Plate 2. a) by adding the commercial grade of NaCl. The NaCl was added slowly until the selected level of salinity achieved. The salinity level was measured by salinity meter. The measured salinity is 30 ppt. Brine solution was minimized into different salinity level for the specific treatment (0, 3, 6, 9, 12, 15, 18 and 21‰) by adding tap water. This brine solution was also used for sequential changing of water (Plate 2.b) of the aquariums for every 7 days at 60 days rearing period.

2.6 Aquariums

The experiment was conducted in 14 aquariums (Plate 2. c) collected from nearer market and arranged them according to experimental design. There were 8 sets of aquariums (2 replica in each set and 8 sets= 1control + 7 treatments) were arranged at the Aquatic lab. The experiment was carried out in glass aquariums having the capacity of 60 L of water and stocked with 10 fish in each. The replacement of water from each aquarium was done for every 7 days by siphoning (Plate 2. d) the bottom of the aquariums at 60 days rearing period.

2.7 Aeration System

Metabolism and biochemical composition of tissues of fishes can drastically changed for the poor dissolved oxygen in the water (Parry, 1966). For the proper aeration of aquariums and acclimatization tank a good aeration system was developed by an electric motor (Plate 3.a). Plastic pipes (plate 3.b); aerator stones were properly arranged in this system.

2.8 Feeding Trial

After completing the preparation of the experimental aquariums, fish fingerlings were released in different aquariums in different salinities and gave them time to adopt with the new environment. The individual weight and length of fish was taken by using electric weight balance (Plate 3. c) and length scale (Plate 3. d) respectively before released. The fish fed with fish feed (plate 4.a) meal of 50% crude protein (CP) at 5% of total body weight. After 3 days of final acclimatization required amount of feed (Plate 4. b) were given two times in a day into the experimental aquariums. Fish weight and length were recorded to find out the Feed efficiency, Feed conversion Ratio, Average growth rate and Specific growth rate of *A. testudineus*

2.9 Survival Trial

To determine the effect of salinity on survival of *Anabas testudineus*, individuals (Plate 4.c) were exposed into 0, 3, 6, 9, 12, 1, 18 and 21‰ salinity under two replica for 60 days rearing period in the Aquatic Laboratory of Department of Fisheries, University of Dhaka. For the purpose of conditioning there was 300 L static rectangular plastic tank was used. At first the fish fingerlings were released into the plastic tank at 0‰ salinity. After 48 hours of acclimatization 10 fish fingerling were released at the 0‰ labeled aquarium and the rest of the fingerlings were kept inside the plastic tank at 3‰ salinity for the next 48 hours. After another 48 hours of acclimatization 10 fish fingerlings were released at the 3‰ labeled aquarium and the rest of the fingerlings were again kept inside the plastic tank at 6‰ saline water for the next 48 hours. By following this procedure the salinity was raised up to 21‰. Finally the mortality and response of the fishes were observed at different salinities.

2.10 Growth Trial

2.10.1 Fish sampling Procedure

Sampling was accomplished at the 15^{th}, 30^{th}, 45^{th} and 60^{th} day of the experimental period. Prior of weighing, the fishes were caught with a fine mesh scoop net (Plate 4.d) and their individual length and weight were recorded.

After 60 days of rearing or at the termination of the experiment, the final length (cm) and weight (g) of the individual fish was carefully recorded. A wooden measuring scale was employed for measuring the lengths. The total body weight of individual fish was determined by a sensitive electronic balance.

2.10.2 Analysis of experimental data

Experimental data which collected during the growth trial were used to determine the following growth parameters.

2.10.2.1 Average growth rate (AGR)

Average growth rate means the increase of body weight per day. It was calculated by the following formula as suggested by Jones (1967).

$$AGR = (W_2 - W_1) / (T_2 - T_1)$$

Where,

T_2= Final time

T_1= Initial time

W_1= Initial mean weight

W_2= Final mean weight

2.10.2.2 Specific growth rate (SGR %)

SGR Mean the percentage of body weight increase per day. Specific growth rate was calculated by the following formula as suggested by Hopkins (1992).

$$SGR\ (\%) = (\ln W_t - \ln W_1) / (T - t) \times 100$$

Where,

In Wt= Natural log of weight at time T

In W_1= Natural log of initial weight

T= Final time

t= Initial time.

2.10.2.3 Feed conversion ratio (FCR)

The FCR is simply the amount of feed it takes to grow a kilogram of fish. Feed conversion ratio (FCR) was determined by the following formula as suggested by Payne (1987).

$$FCR = \text{Feed (g) consumed by the fish} / (W_2 - W_1)$$

Where,

W_2= Final weight

W_1=Initial weight

2.11 Statistical analysis

All the structured designs and data were analyzed using a one-way ANOVA. This included significant results ($p<0.05$) were taken as rejection of the null hypothesis which showed significance differences between the treatments. These results are displayed as superscripts against each respective value. All statistical analyses were carried out using Microsoft excel program (version 2007) and SPSS (Statistical Package for the Social Sciences) program (version 11.5).

LIST OF PLATES

a

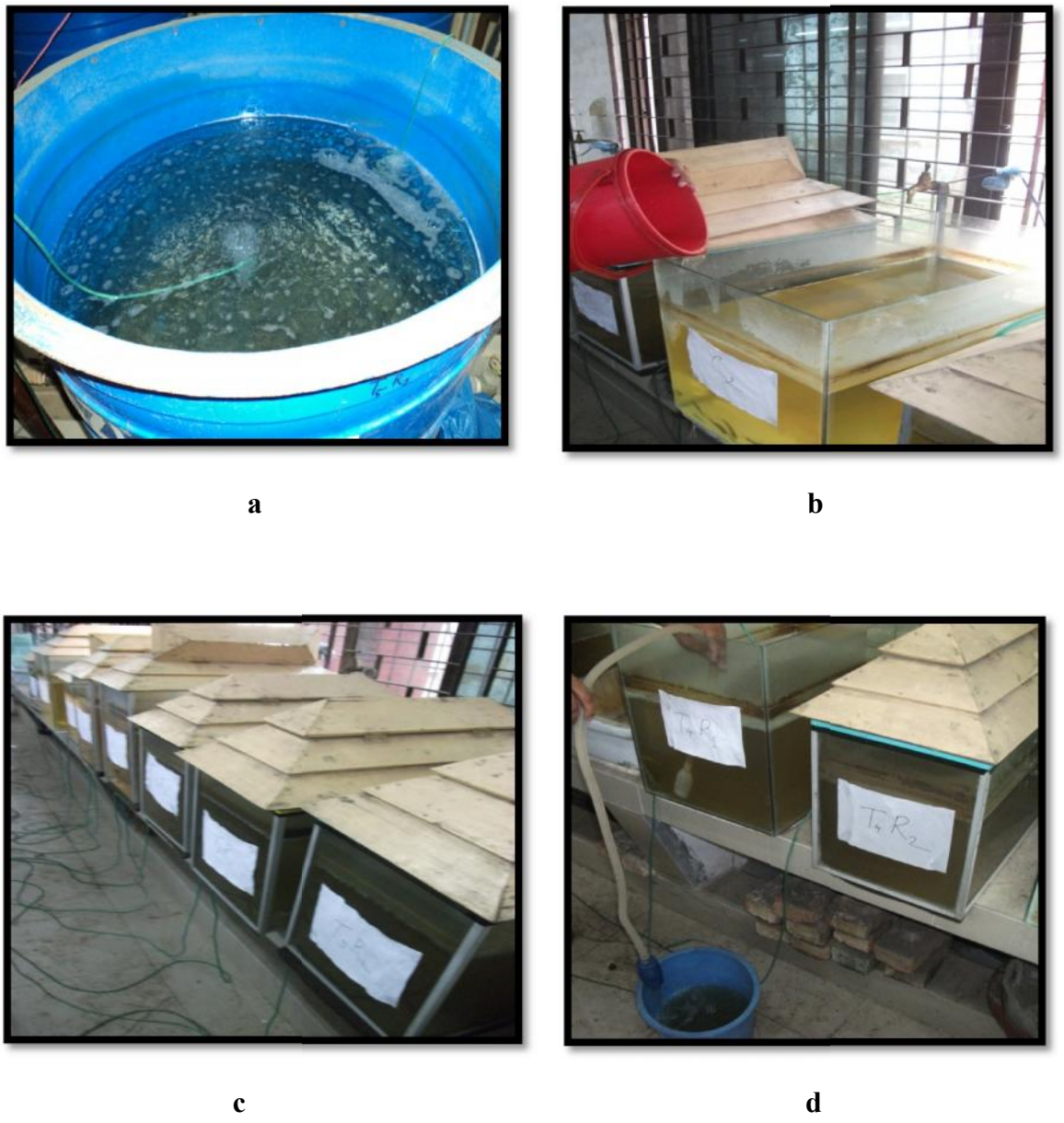
b

c

d

Plate 1. Salinity meter (a), pH Meter (b), Dissolved Oxygen, DO meter (c), Fish are being acclimatized in acclimatization Tank (d).

LIST OF PLATES



a **b**

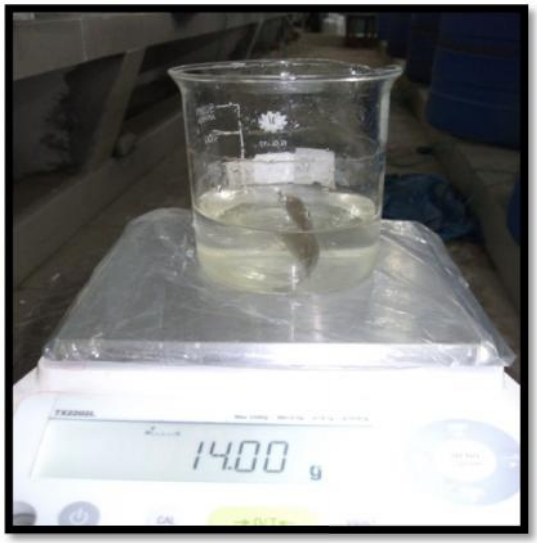
c **d**

Plate 2. Brine solution tank (a), Addition of water (b), Experimental aquarium (c), Siphoning for releasing water and bottom dust (d).

LIST OF PLATES

a

b



c

d

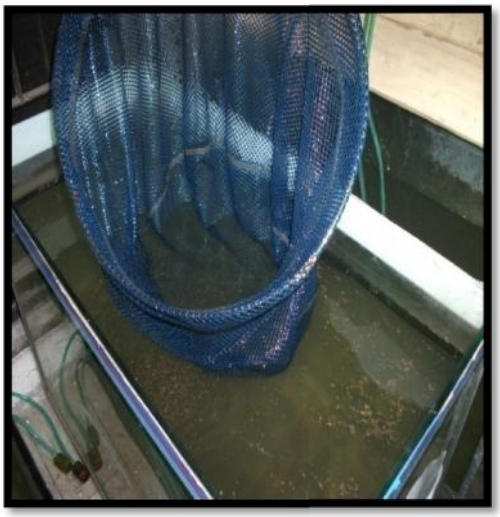
Plate 3. Electric motor (a), Aeration of aquarium water (b), Electric weight balance (c), Length scale (d).

LIST OF PLATES

a

b

c



d

Plate 4. Fish feed (a), Giving feed in aquarium (b), Fish in different treatments (c), Scoop net for sampling (d).

Chapter 03
Results

CHAPTER 03
RESULT

3.1 Effects of salinity on the survival rate of *Anabas testudineus.*

Anabas testudineus fingerlings showed 100% survival rate between 0 to 9‰ salinities, 60% survival rate in 12‰ salinities, 20% survival rate in 15‰ salinities and all death (100% mortality) in 18 and 21‰ salinities (Figure 1).

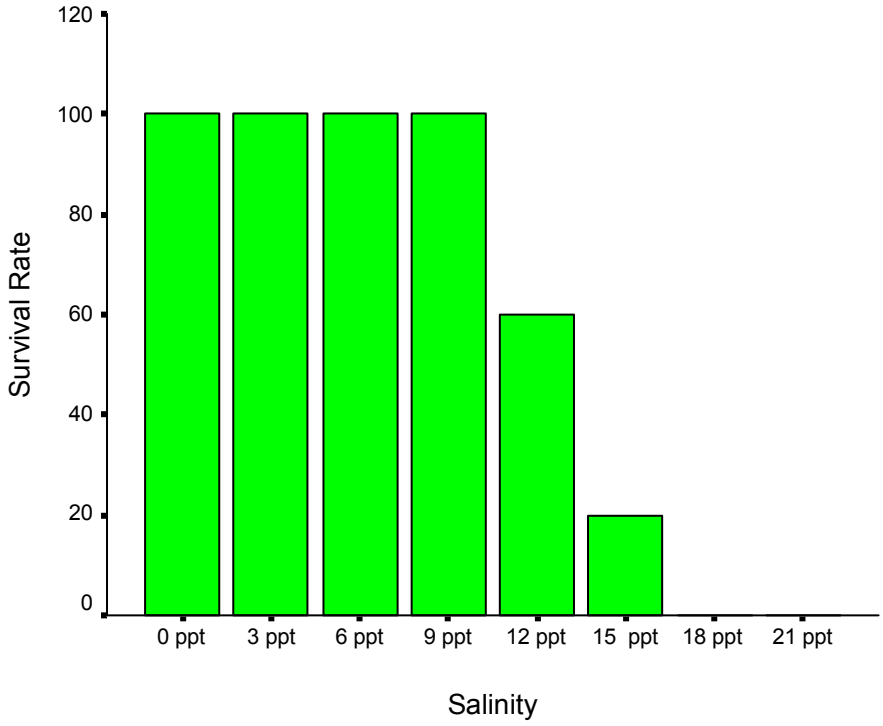


Figure 1. Survival rate of *Anabas testudineus* in different salinities

During 60 day rearing period all fingerlings survived in 0 to 9‰ salinities. In 12‰ salinity survival rate was 85% at 15 days rearing period and then decreased up to 60% at 60 days rearing period. At 15‰ salinity survival rate was 75% at 15 days rearing period and then decreased up to 20% at 60 days rearing period. At 18‰ salinity survival rate was 100% up to 2 days and then dropped into 20% at 3rd day and 0% (100% mortality) at 4th day. In 21 ‰ salinity 100% survival was recorded at 1st day but 0% (100% mortality) at next day (Table 1).

Table 1. Survival rate (%) of *Anabas testudineus* fingerlings at different salinity (‰) level in 60 day rearing period. Individual letters denote significantly different.

Salinity (‰)	Survival Rate			
	15 day	30 day	45 day	60 day
0	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
3	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
6	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
9	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}	100 ± 0.0^{a}
12	85.0 ± 5.0^{ab}	80.0 ± 0.0^{bc}	67.5 ± 2.5^{bcd}	60.0 ± 10.0^{cde}
15	75.0 ± 5.0^{bcd}	55.0 ± 5.0^{de}	40.0 ± 10.0^{ef}	20.0 ± 5.0^{f}

[1]Values are mean ± SEM of duplicate groups of 10 fish. Means in the same column with different superscripts are significantly different at $P<0.05$.

3.2 Feed conversion ratio

Increasing trends of feed conversion ratio (FCR) was found with increasing salinity. However, highest FCR was recorded at 15‰ salinity and lowest FCR was recorded at 0‰ (Figure 2).

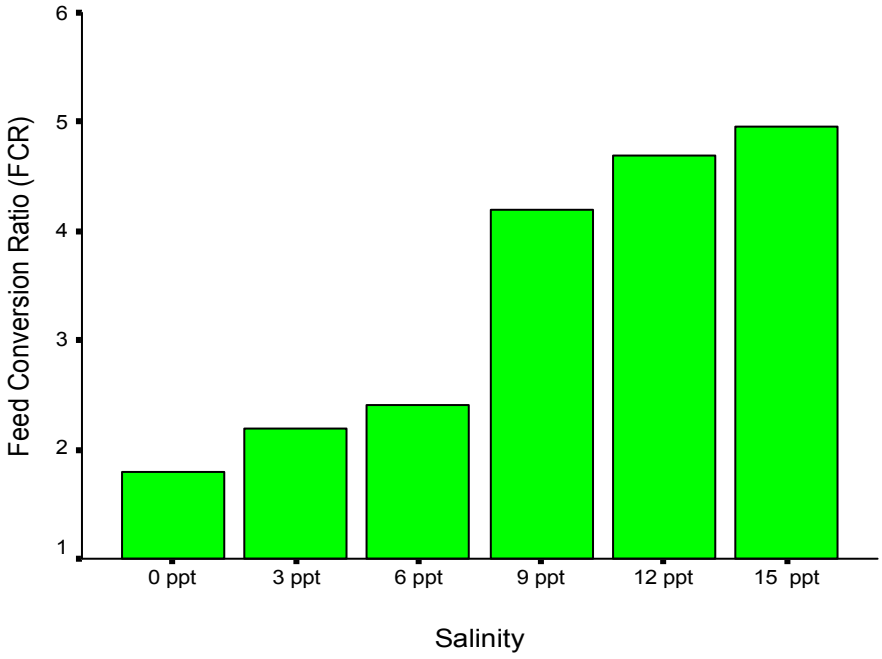


Figure 2. Feed conversion ratio (FCR) of *Anabas testudineus* in different salinities.

3.2.1 Within treatment

Significantly lower FCR was found in *A. testudineus* fingerlings reared at 0‰ salinity at 60 day (1.55 ± 0.05^{e}) than that of other days while similar FCR was observed at the same salinity at 15, 30 and 45 day rearing period. Moreover, Significantly lower FCR was observed in *Anabas* fingerlings reared at 3‰ and 6‰ salinity at 60 day than that of other days while similar FCR was found at the respective salinity at 15, 30 and 45 day rearing period. Besides, significantly higher FCR was detected in *A. testudineus f*ingerlings reared at 9‰ salinity at 15 day (4.35 ± 0.05^{ab}) than that of other days while similar FCR was observed at the same salinity at 30, 45 and 60 day rearing period. There was significantly higher FCR was detected in *A. testudineus* fingerlings reared at 12‰ salinity at 60 day (4.70 ± 0.20^{ab}) than that of other days while similar FCR was observed at the same salinity at 15, 30 and 45 day rearing period. Similarly significantly higher FCR was detected in *A. testudineus* fingerlings reared at 15‰ salinity at 45 day (5.20 ± 0.20^{a}) than that of other days while similar FCR was observed at the same salinity at 15, 30 and 60 day rearing period (Table 2).

3.2.2 Within duration

Significantly lower FCR was detected in *A. testudineus* fingerlings reared at 15 day duration at 0‰ to 6‰ salinity than that of other salinity while similar FCR was observed at the same duration at 9, 12 and 15‰ salinity. Moreover significantly lower FCR was found in *A. testudineus* fingerlings reared at 30, 45 and 60 day duration at 0‰ to 6‰ salinity than that of other salinity while similar FCR was found at the respective same duration at 9, 12 and 15‰ salinity. However the lowest FCR was found at 60 day (1.55 ± 0.05^{e}) duration at 0‰ and highest FCR was evaluated at 45 day (5.20 ± 0.20^{a}) duration at 15‰ (Table 2).

Table 2. Feed conversion ratio, FCR (Mean ± SEM)[1] of *Anabas* testudineus fingerlings at 60 day rearing period. Individual letters denote significantly different.

Salinity (‰)	Feed Conversion Ratio (FCR)			
	15 day	30 day	45 day	60 day
0	1.75 ± 0.05^{e}	2.00 ± 0.10^{cde}	1.64 ± 0.46^{de}	1.55 ± 0.05^{e}
3	2.30 ± 0.10^{cde}	1.85 ± 0.05^{e}	2.75 ± 0.35^{cd}	1.90 ± 0.00^{de}
6	2.35 ± 0.05^{cde}	2.40 ± 0.20^{cde}	2.80 ± 0.10^{c}	2.05 ± 0.15^{cde}
9	4.35 ± 0.05^{ab}	4.15 ± 0.35^{b}	4.10 ± 0.10^{b}	4.20 ± 0.10^{b}
12	4.80 ± 0.05^{ab}	4.70 ± 0.10^{ab}	4.60 ± 0.00^{ab}	4.70 ± 0.20^{ab}
15	4.85 ± 0.05^{ab}	4.80 ± 0.10^{ab}	5.20 ± 0.20^{a}	4.95 ± 0.25^{ab}

[1]Values are mean ± SEM of duplicate groups of 10 fish. Means in the same column with different superscripts are significantly different at $P<0.05$.

3.3 Specific growth rate

Specific growth rate (SGR) was decreased as the salinity increased. The highest SGR was recorded at 0‰ salinity and lowest (SGR) was recorded at 15‰ salinity (Figure 3).

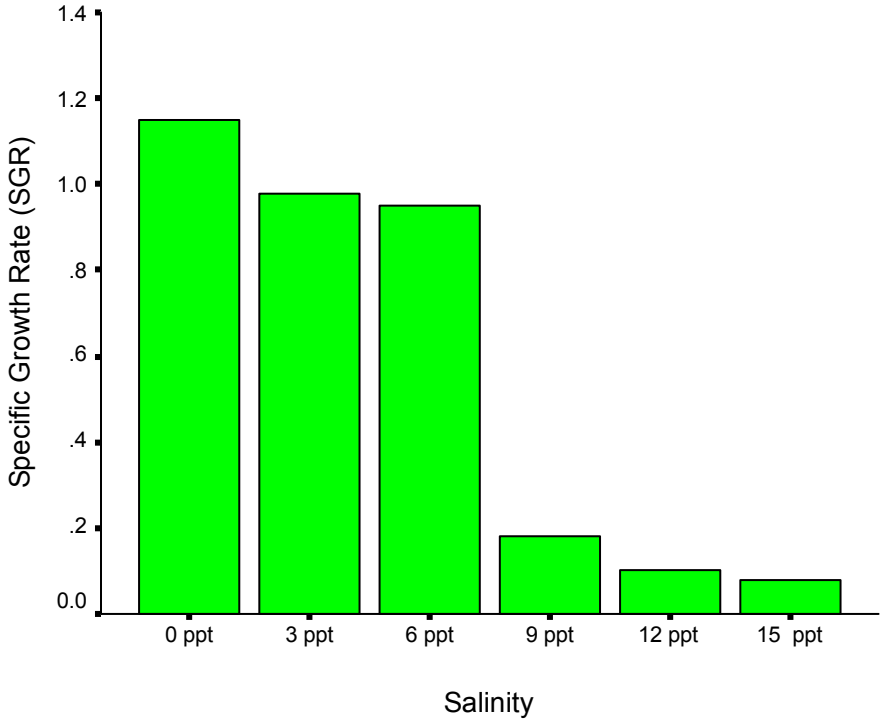


Figure 3. Specific growth rate (SGR) of *Anabas testudineus* in different salinities.

3.3.1 Within treatment

There was higher SGR was found in *A. testudineus* fingerlings reared at 0‰ salinity at 60 day (1.16 ± 0.05^{a}) than that of other days while similar SGR was observed at the same salinity at 15, 30 and 45 day rearing period which was significant. Moreover, Significantly higher SGR was observed in *A. testudineus* fingerlings reared at 3‰ and 6‰ salinity at 60 day than that of other days while similar SGR was found at the respective salinity at 15, 30 and 45 day rearing period. Besides, significantly lower SGR was detected in *A. testudineus* fingerlings reared at 9‰ salinity at 15 and 30 day (0.17 ± 0.01^{cd}) than that of other days while similar SGR was observed at the same salinity at 45 and 60 day rearing period. Significantly lower SGR was detected in *A. testudineus* fingerlings reared at 12‰ salinity at 30 and 45 day (0.08 ± 0.01^{cd}) than that of other days while similar SGR was found at the same salinity at 15 and 60 day rearing period. Similarly significantly lower SGR was detected in *A. testudineus* fingerlings reared at 15‰ salinity at 30 day (0.07 ± 0.03^{d}) than that of other days while similar SGR was observed at the same salinity at 15, 45 and 60 day rearing period (Table 3).

3.3.2 Within duration

Higher SGR was observed in *A. testudineus* fingerlings reared at 15 day duration at 0‰ to 6‰ salinity than that of other salinity while similar SGR was found at the same duration at 9, 12 and 15‰ salinity which was significant. Moreover significantly higher SGR was found in *A. testudineus* fingerlings reared at 30, 45 and 60 day duration at 0‰ to 6‰ salinity than that of other salinity while similar SGR was detected at the respective same duration at 9, 12 and 15‰ salinity. However the lowest SGR was observed at 30 day (0.07 ± 0.03^{d}) duration at 15‰ and highest SGR was evaluated at 60 day (1.16 ± 0.05^{a}) duration at 0‰ (Table 3).

Table 3. Specific growth rate, SGR (Mean ± SEM)[1] of *Anabas testudineus* fingerlings at 60 day rearing period. Individual letters denote significantly different.

Salinity (‰)	Specific Growth Rate (SGR)			
	15 day	30 day	45 day	60 day
0	1.15 ± 0.02^{a}	1.14 ± 0.02^{a}	1.14 ± 0.05^{a}	1.16 ± 0.05^{a}
3	0.97 ± 0.01^{b}	0.99 ± 0.01^{b}	0.96 ± 0.02^{b}	0.99 ± 0.00^{b}
6	0.96 ± 0.02^{b}	0.94 ± 0.03^{b}	0.93 ± 0.01^{b}	0.96 ± 0.01^{b}
9	0.17 ± 0.01^{cd}	0.17 ± 0.01^{cd}	0.18 ± 0.01^{c}	0.18 ± 0.01^{c}
12	0.12 ± 0.01^{cd}	0.08 ± 0.01^{cd}	0.08 ± 0.01^{d}	0.11 ± 0.01^{cd}
15	0.07 ± 0.01^{d}	0.07 ± 0.03^{d}	0.08 ± 0.00^{d}	0.09 ± 0.00^{cd}

[1]Values are mean ± SEM of duplicate groups of 10 fish. Means in the same column with different superscripts are significantly different at $P<0.05$.

3.4 Average Growth Rate (gday^{-1})

The average growth rate (AGR) of *Anabas testudineus* was similarly decreased like SGR as the salinity increased. Highest AGR was evaluated at 0‰ salinity and lowest was 15‰ salinity (Figure 4).

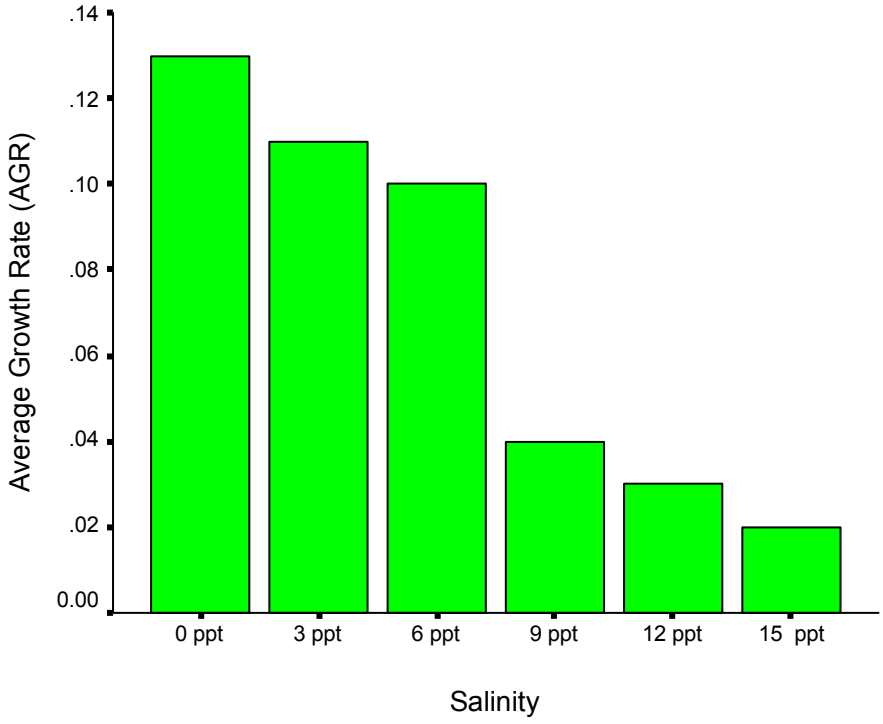


Figure 4. Average growth rate (AGR) of *Anabas testudineus* in different salinities.

3.4.1 Within treatment

Significantly higher AGR was recorded in *A. testudineus* fingerlings reared at 0‰ salinity at 60 day (0.14 ± 0.01^{a}) than that of other days while similar AGR was observed at the same salinity at 15, 30 and 45 day rearing period. Moreover, Significantly higher AGR was observed in *A. testudineus* fingerlings reared at 3‰ and 6‰ salinity at 60 day than that of other days while similar AGR was found at the respective salinity at 15, 30 and 45 day rearing period. Besides, significantly lower AGR was detected in *A. testudineus* fingerlings reared at 9‰ salinity at 30 and 45 day (0.03 ± 0.01^{def}) than that of other days while similar AGR was observed at the same salinity at 45 and 60 day rearing period. Significantly lower AGR was detected in *A. testudineus* fingerlings reared at 12‰ salinity at 45 day (0.03 ± 0.02^{def}) than that of other days while similar AGR was found at the same salinity at 15, 30 and 60 day rearing period. Similarly significant lower AGR was detected in *A. testudineus* fingerlings reared at 15‰ salinity at 45 day (0.01 ± 0.01^{f}) than that of other days while similar AGR was observed at the same salinity at 15, 30 and 60 day rearing period (Table 4).

3.4.2 Within duration

Higher AGR was observed in *A. testudineus* fingerlings reared at 15 day duration at 0‰ to 6‰ salinity than that of other salinity while similar AGR was found at the same duration at 9, 12 and 15‰ salinity which was significant. Moreover significantly higher AGR was found in *A. testudineus* fingerlings reared at 30, 45 and 60 day duration at 0‰ to 6‰ salinity than that of other salinity while similar AGR was detected at the respective same duration at 9, 12 and 15‰ salinity. However the lowest AGR was observed at 45 day (0.01 ± 0.01^{f}) duration at 15‰ and highest AGR was evaluated at 60 day (0.14 ± 0.01^{a}) duration at 0‰ (Table 4).

3.5 Effect of salinity on feeding response of *Anabas testudineus.*

A. testudineus fingerlings showed very high appetitive behavior to food between 0 to 6‰ salinities in 60 day rearing period. At 9‰ salinity fingerlings showed very high appetitive behavior within almost half of the experiment (27 days) and then showed high appetitive behavior from 29 to 45 days and low appetitive behavior showed at the last 15 days (45 to 60 days) of the experiment. The fingerlings showed high appetitive behavior at the first 9 days of the experiment, showed then moderate appetitive behavior up to 33 days and low appetitive behavior showed at the remaining days at the 12‰ salinity.

Table 4. Average growth rate, AGR (Mean ± SEM)[1] of *Anabas* testudineus fingerlings at 60 day rearing period. Individual letters denote significantly different.

Salinity (‰)	Average Growth Rate (AGR)			
	15 day	30 day	45 day	60 day
0	0.13 ± 0.01^{a}	0.12 ± 0.01^{a}	0.11 ± 0.03^{ab}	0.14 ± 0.01^{a}
3	0.11 ± 0.01^{abc}	0.10 ± 0.01^{abcd}	0.10 ± 0.01^{abcd}	0.12 ± 0.02^{ab}
6	0.09 ± 0.01^{abcde}	0.09 ± 0.03^{abcde}	0.09 ± 0.01^{abcde}	0.12 ± 0.01^{ab}
9	0.05 ± 0.01^{bcdef}	0.03 ± 0.03^{def}	0.03 ± 0.01^{def}	0.04 ± 0.01^{cdef}
12	0.02 ± 0.01^{ef}	0.03 ± 0.00^{ef}	0.03 ± 0.02^{def}	0.02 ± 0.01^{ef}
15	0.03 ± 0.00^{ef}	0.02 ± 0.03^{ef}	0.01 ± 0.01^{f}	0.01 ± 0.00^{f}

[1]Values are mean ± SEM of duplicate groups of 10 fish. Means in the same column with different superscripts are significantly different at $P<0.05$.

At 15‰ salinity started with moderate appetitive behavior for first 11 days and then showed low appetitive behavior for the remaining duration. At 12 and 15‰ salinity levels sequential death was occurred due to osmoregulatory failure. *A. testudineus* fingerlings showed low appetitive behavior from first day of the experiment and then 100% death was occurred within 4 days and 2 days at 18‰ and 21‰ salinity level respectively (Table 5).

3.6 Effect of salinity on threat response of *Anabas testudineus.*

The fish exhibited a normal response to threat between 0 to 9‰ salinity levels. At 12‰ salinity first 15 days showed normal response and next 30 days showed hyper activeness and death was recorded at 45 day of rearing period. In 15‰ salinity moderate response showed at first five days hyper activeness showed at next five days and death was started from 13 days. At 18‰ salinity *A. testudineus* fingerlings showed hyper activeness at first two days and all death within four days. At 21‰ salinity death was recorded from very first day and all death within 2 days (Table 6).

Table 5. Summary of daily feeding response of *Anabas testudineus* in different salinity regimes.

Day	Salinity concentration (‰)							
	0	3	6	9	12	15	18	21
01	VHA	VHA	VHA	VHA	HA	MA	LA	LA
03	VHA	VHA	VHA	VHA	HA	MA	LA	D
05	VHA	VHA	VHA	VHA	HA	MA	D	D
07	VHA	VHA	VHA	VHA	HA	MA	D	D
09	VHA	VHA	VHA	VHA	HA	MA	D	D
11	VHA	VHA	VHA	VHA	MA	MA	D	D
13	VHA	VHA	VHA	VHA	MA	LA	D	D
15	VHA	VHA	VHA	VHA	MA	LA	D	D
17	VHA	VHA	VHA	VHA	MA	LA	D	D
19	VHA	VHA	VHA	VHA	MA	LA	D	D
21	VHA	VHA	VHA	VHA	MA	LA	D	D
23	VHA	VHA	VHA	VHA	MA	LA	D	D
25	VHA	VHA	VHA	VHA	MA	LA	D	D
27	VHA	VHA	VHA	VHA	MA	LA	D	D
29	VHA	VHA	VHA	HA	MA	LA	D	D
31	VHA	VHA	VHA	HA	MA	LA	D	D
33	VHA	VHA	VHA	HA	MA	LA	D	D
35	VHA	VHA	VHA	HA	LA	LA	D	D
37	VHA	VHA	VHA	HA	LA	LA	D	D
39	VHA	VHA	VHA	HA	LA	LA	D	D
41	VHA	VHA	VHA	HA	LA	LA	D	D
43	VHA	VHA	VHA	HA	LA	LA	D	D
45	VHA	VHA	VHA	LA	LA	LA	D	D
47	VHA	VHA	VHA	LA	LA	LA	D	D
49	VHA	VHA	VHA	LA	LA	LA	D	D
51	VHA	VHA	VHA	LA	LA	LA	D	D
53	VHA	VHA	VHA	LA	LA	LA	D	D
55	VHA	VHA	VHA	LA	LA	LA	D	D
57	VHA	VHA	VHA	LA	LA	LA	D	D
60	VHA	VHA	VHA	LA	LA	LA	D	D

VHA= Very High Appetite, HA= High Appetite, MA= Moderate Appetite, LA= Low Appetite, D= Death

Table 6. Summary of threat response of *Anabas testudineus* in different salinity regimes

Day	Salinity concentration (‰)							
	0	3	6	9	12	15	18	21
01	N	N	N	N	N	M	H	D
03	N	N	N	N	N	M	H	D
05	N	N	N	N	N	M	D	D
07	N	N	N	N	N	H	D	D
09	N	N	N	N	N	H	D	D
11	N	N	N	N	N	H	D	D
13	N	N	N	N	N	D	D	D
15	N	N	N	N	N	D	D	D
17	N	N	N	N	H	D	D	D
19	N	N	N	N	H	D	D	D
21	N	N	N	N	H	D	D	D
23	N	N	N	N	H	D	D	D
25	N	N	N	N	H	D	D	D
27	N	N	N	N	H	D	D	D
29	N	N	N	N	H	D	D	D
31	N	N	N	N	H	D	D	D
33	N	N	N	N	H	D	D	D
35	N	N	N	N	H	D	D	D
37	N	N	N	N	H	D	D	D
39	N	N	N	N	H	D	D	D
41	N	N	N	N	H	D	D	D
43	N	N	N	N	H	D	D	D
45	N	N	N	N	D	D	D	D
47	N	N	N	N	D	D	D	D
49	N	N	N	N	D	D	D	D
51	N	N	N	N	D	D	D	D
53	N	N	N	N	D	D	D	D
55	N	N	N	N	D	D	D	D
57	N	N	N	N	D	D	D	D
60	N	N	N	N	D	D	D	D

N= Normal Response, M= Moderate Response, H= Hyperactive, D= Death

Chapter 04
Discussion

CHAPTER 04
DISCUSSION

Anabas testudineus having high consumer demand in Bangladesh showed different survival rate at different salinity regime. Findings of the study showed that *A. testudineus*, tolerated between 0 to 9‰ salinity regime. In these regimes no mortality were recorded. High growth performances in terms of total length (TL), body weight (BW), specific growth rate (SGR), feed conversion ratio (FCR) and average growth rate (AGR) were recorded between 0 to 6‰ salinity level. This is an indication that the fish were perfectly able to regulate their body physiology within this regime. 100% mortality was recorded in 18 and 21‰ salinity which indicates the developed osmo-regulatory failure in fish. The mortality might be due to stress, duress and less resistance of the fish to these salinities.

Survival rate of 100% between 0 to 9‰ salinity reveals that the fish can withstand an intermediate salinity range which may be because of the ability of the body fluids to function at least for short time in an abnormal range of internal osmotic and ionic concentrations. The fish can regulate the body fluid to restore level of osmotic pressure to near normal (Kurata, 1959; Holliday and Jones, 1967). Tarer (2000), also recorded significant difference in *Labeo rohita* and *Cirrhinus mrigala* in tolerance of salinity and found *Labeo rohita* (Rohu) more tolerant towards salinity up to 7‰ which supports the present findings.

Nelson (1987) also found similar results while working on salinity tolerance of different freshwater fish species. Kasim (1983) found variable behavioral patterns in different carps at different salinity, where common carp showed higher tolerant to salinity than that of rohu and mirigal. Kliambi and Zdinak (1980), while working with grass carp fingerlings recorded between 71-90% mortality in 24 h under 15‰ salinity. Nugon (2003) reports on juveniles of *O. aureus*, *O. niloticus* and Florida red Tilapia showed they exhibited good survival (>81%) in salinity regimes up to 20‰, with moderate survival of *O. aureus* (54%) and Florida red tilapia (33%) at 35‰ salinity. The commercial carp survived salinity regimes up to 8‰ but exhibited poor survival (5%) at 15‰ salinity.

Feed conversion ratio values found in fish reared at 0, 3 and 6‰ indicates good growth rate. Better FCR (1.55 ± 0.05^{e}) showed 0‰ salinity at 60 day. The efficiency of feed conversion depends on many factors but the best response is probably strongly related to optimize the environment to approximate that to which the fish is accustomed (Canagaratnam, 1966). Otto (1971) found that *Oncorhynchus kisutch* growth rate, feed intake and feed conversion efficiency had the highest values at salinities 5 to 10‰. For rainbow trout (*Oncorhynchus mykiss),* the gross diet efficiency was not significantly affected by the environmental salinity (Zeitoun *et al.*, 1973). Doolgindachabaporn (1994) found that the FCR value of *Cyprinous carpio* ranges from 1.8 to 3.0 and Akand *et.al*. 1989 found FCR value 2.0 to 2.7 in case of *Heteroneustes fossilis.*

In terms of Specific growth rate (SGR) the highest SGR (1.16 ± 0.05^{a}) was detected at 0‰ at 60 day duration, but SGR decreased with increasing salinity. This finding resembles the Medawars (1945) fifth law "the specific growth rate declines more and more slowly as the organism increases in age". Minot (1990) was the first person to recognize that for most animals the specific growth rate is highest early in life and that it typically decreases with increasing age, becoming zero in some animals.
Fishes usually have a range of requirements (i.e., temperature, DO, salinity) for a growth and successful spawn, and if one variable is not within acceptable limits, reproduction may not be successful and growth not occur properly (Barnard and McBain, 1994; Wedemeyer, 2001). Pike silverside, *Chrostoma estor* can tolerate salinity up to 5‰ but SGR and survival reduced at salinities of 10‰ and above (Martinez-Palacios *et al.*, 2004; Martinez-Palacios *et al.*, 2008). Salinity can also affect SGR of *Common carp* and tend to be zero at the adult (Altinok and Grizzle, 2004). The SGR of juvenile alligator gar was not affected by salinity; however, FCR was lowest at 0‰. Altinok and Grizzle (2004) found that three freshwater euryhaline species had better SGR and FCR at 1 to 6‰ salinity compared to 7 to 13‰ salinity level.

Anabas testudineus shown nearly similar average growth rate (AGR) between 0 to 6‰ salinities at 60 days rearing period. The maximum AGR (0.14 ± 0.01^{a}) was recorded at 0‰ salinity a 60 day and lower AGR (0.01 ± 0.01^{f}) was found in 15‰ salinity at 45 day. Higher AGR of the fish suggested that the fish were able to regulate osmotic pressure of the body fluid; this was in agreement with suggestions of Nikolsky (1963), the more the osmo-regulatory adaptation, lesser the difference between the compositions and pressures of the internal fluid of the organism and its external environment. The migration of fish from fresh to seawater will normally lead to increase osmotic concentration of fish's blood serum and change in ionic contents and decrease the rate of daily gain (Gordon, 1959; Miles and Smith, 1968). Job (1969) suggested that at 10‰ salinity *Oreochromis mossambicus* was able to osmoregulate more efficiently with a consequent daily gain (Febry and Lutz, 1987).

The restlessness or hyper-activeness or erratic behavior in high salinities indicates fast rate at which the fish were approaching their tolerance limits and loss of water to external medium from the body. In case of *Anabas testudineus* from this research normal response was found up to 9‰ salinity and then hyper-activeness or erratic behavior or death shown at 12 to 21‰. Fish move to preferred position in salinity gradient, to indicate salinity preferences in choice situation (Baggerman, 1959 and McInerney, 1964). Such changes with increased salinity are indication that the salinities were near or outside the tolerance limits of the fish.

Moderate and normal responses represented the near and far tolerance limits respectively. Hoar and Randal (1969) based the survival of fish on a combination of tissue tolerance and regulation, higher osmo-regulatory cost at higher salinity could make fish to develop body lesions which covered 25% of their body surface. McGeachin *et al.* (1987) and Hopkin *et al.* (1989) observed external lesions and severe hemorrhages in internal organs in *O. aureus* in 10 to 12‰ salinities.

High appetitive behavior of *Anabas testudineus* was recorded between 0 to 6‰ salinity level in this study and sequentially lowered and death occurred at 12 to 21‰. The high appetitive behavior displayed by the fish towards food is an indication that fish body metabolism can still be maintained or regulated in these salinities, while low appetite is an indication of near or total body metabolic break down. Miller (1976), Resendez (1981) and Chavez *et al.* (1983) observed *Oreochromis niloticus* showed high appetitive behavior between 0 to 7‰ salinities. Common carp, *Cyprinus carpio* showed high resistant, better feeding rate and as well as growth up to 6‰ salinity level (Martinez-Palacios and Ross, 1986).

Water temperature has profound effect on growth, reproduction and other biological activities of fishes. In experimental period overall temperature was found 25.10 to 31.20^{o}C. These findings were more or less similar to the findings of Rahman (1982) who reported that the water temperature ranged from 26.06 to 31.97° C was suitable for fish culture. Mollah and Haque (1978) recognized water temperature during their studies and reported the water temperature range 26.06-31.97° C suitable for *Cyprinous carpio*. Rubia and Teng (2005), reported that optimum temperature for fresh water cat fish (*Ictalurus punctatus*) and water pumpkin seed fish (Lepomis *gibbosus*) is 29--30°C. Deacon (1997) reported that the growth rate of African cat fish at 29 °C is higher than at 24 °C.

Dissolved oxygen (DO) is the most important physical factor for all aquatic organisms including fish. Overall DO was 5.5 to 7.95 mg/L during experiment. Claireaux and Lagardere (1999) reported that the dissolved oxygen content of water ranging from 6.7 - 8.3 mg/L was satisfactory level for fish production. The above findings has got similarities within the findings of Mandal (1998) also got the values of DO between 4.7 - 8.4 mg/L. Oxygen levels of 4 mg\L and above have been recommended by Peterson (1999) for satisfactory level of fish culture. Hydrogen ion concentration (pH) is very important factor in fish life. The overall pH value in the present investigation was 8.1 to 9.6. According to Swingle (1957) pH 6.5-9.0 is suitable for pond fish culture.

Chapter 05
Conclusion and Recommendations

CHAPTER 05

CONCLUSION AND RECOMENDATION

5.1 Conclusion

In this study, the significant effects of salinity on survival, behavioral responses and growth performance of *A. testudineus* were investigated. *A. testudineus* fingerlings can adapt to gradual increase of salinity. Normal growth was observed at salinity regimes of 0-6‰. However, the most preferred salinity was 3‰. For aquaculture purpose, the findings of this study suggest that *A. testudineus* fingerlings can be cultured in aquatic environment with salinities between 0-6‰ which confirm high production and better economic return.

5.2 Recommendations

- To increase the production of *A. testudineus* further research should be needed to identify the mechanisms which are involved in the regulation of a response to salinity.
- During the current study interval of salinity was 3‰. Further studies should be conducted by decreasing the salinity interval such as 1 or 2 ‰ salinity.
- In the current study few parameters are selected such as survivability, SGR, FCR, ADG and water quality parameters in different salinities. Further study may be conducted on the hematological profile, histopathological profile and proximate composition of fish in different salinities.
- Need to determine the osmomolality of *A. testudineus* in different salinities.
- Further study on the enzymes that help to regulate the gill activities at saline water can be conducted.
- Current study is totally laboratory based, so field studies will be needed to clarify the actual effect of different salinity level on *A. testudineus*.

References

REFERENCES

AHMED, G. U., ISLAM, M. F., KFIAN, M. N. A., HAQUE, M. M AND KIBRIA, M. G., 1997. Culture feasibility of African catfish (*Clarias gariepinus*) fry in glass tank and synthetic hapa system using supplemental diets. Bangladesh Journal of Fisheries Research**, 2:** 55-66.

AHMED, G. U., MULLAH, M. F. A., KARIM, A AND SARDER, M. R. I., 1991. Effects of aeration on growth and survival of *Clarias gariepinus* larvae under culture. Bangladesh Journal of Fisheries Research, **l4:** 3l-35.

AKAND, A. M., MIA, M. L AND HAQUE, M. M., l989. Effect of dietary protein level on growth, food conversion and body composition of Shingi, *Heteropneustes fossilis*. Aquaculture**, 77:** 175-180.

ALABASTER, J. S AND LLOYD, R., 1982. Water Quality Criteria for Freshwater Fish. Aquaculture**, 4:** 355- 361.

ALAM, M. S AND MOLLAH, M. F. A., 1998. Formulation of an artificial dry feed for primary nursing of catfish (*Clarias batrachus*) larvae. Bangladesh Journal of Fisheries Research, **11:** 70-75.

ALAVA, V. R., 1998. Effects of salinity, dietary lipid source and level on growth of milkfish (*Chanos chanos*) fry. Aquaculture, **167:** 229-236.

ALBASTER, J. S AND LIOYD, R., 1980. Oxygen consumption of two marine teleosts, *Lithognathus mormyrus* and *Lithognathus lithognathus*. Comparative Biochemistry and Physiology,vol. **85A** (2): 313-331.

ALI, R. T., CROCKER, P. A., ARNOLD, C. R., DE BOER, J. A AND HOLT, J. D., 1982. Preliminary evaluation of survival and growth of juvenile red drum (*Sciaenops ocellata*) in fresh and salt water. Journal of the World Mariculture Society, **12:** 12-134.

ALI, W. T, DE LA GANDARA, F., GARCIA-GOMEZ, A AND JOVER, M., 1980. Effects of feeding frequency on the daily oxygen consumption rhythms in young Mediterranean yellowtails (*Seriola dumerili*). Aquacultural Engineering, **26:** 9-39.

ALIKUNHI, T., 1957. Osmotic and ionic regulation in captive fish with particular reference to salmonids. Comparative Biochemistry and Physiology,vol. **73 B:** 125-141.

ALTINOK, I AND GRIZZLE, J. M., 2004. Excretion of ammonia and urea by phylogenetically diverse fish species in low salinities. Aquaculture**, 238**: 499-507.

APHA, 1998. Standard Methods for the Examination of Water and Wastewater. Journal of American Public Health Association, **238:** 399-407.

ARUNACHALAM, S AND REDDY, S. R., 1979. Feed intake, growth, feed conversion and body composition of catfish exposed to different salinities. Aquaculture, **16:** 229-238.

AUDET, C., BESNER, M., MUNRO, J AND DUTIL, J. D., 1993. Seasonal and duel variations of various blood parameters in Atlantic cod (*Gadus morhua*) and

plaice (*Hippoglossoides platessoides*). Canadian Journal of Zoology, **71:** 611-618.

BACELA, N., 2002. Studies on captive rearing of spotted grunter *Pommadasys commersonnii* under ambient conditions. Aquacultural Engineering**, 21:** 87-99.

BAGGERMAN, B., 1959. The Role of External Factors and Hormones in Migration of Sticklebacks and Juvenile Salmon. Journal of Comparative Endocrinology**, 8:** 9-37.

BANERJEA, H., 1967. On the relation between the temperature and the respiratory exchange in fishes. International Review of Hydrobiology and Hydrography, **7:**3-5.

BARNARD, T AND MCBAIN, W., 1994. Salt and water balance in rainbow trout (*Salmo gairdneri)* rapidly transferred from fresh water to sea water. Journal of Experimental Biology. **83**, 193– 202.

BAYNE, B. A., 1975. Aspects of physiological condition in *Mytilus edulis* with special reference to the effects of oxygen tension and salinity. European Marine Biology,vol.**4:** 213-238.

BESRA, T. M., 1997. An atlas of Distribution of the Freshwater Fish Families of the World University of Nebraska Press, Lincoln, **4:** 190-197pp

BOEUF, G AND PAYAN, P., 2001. How should salinity influence growth? Comparative Biochemistry and Physiology,vol. **130:** 411-42.

BOONYARATPALIN, M., 1997. Nutrient requirements of marine feed fish cultured in South East Asia. Aquaculture **151:** 283-313.

BRETT, J. R AND GROVES, T. D. D., 1979. Physiological uspets changed due to salinity and temperature at salt lake. Journal of Hydrobiology**, 82 :**162- 352.

BUCKEL, J. A., STEINBERG, N. D AND CONOVER, D. O., 1995. Effects of temperature, salinity, and fish size on growth and consumption of juvenile bluefish. Journal of Fish Biology**, 47:** 696-706.

BUREL, C., PERSON-LE RUYET, J., GAUMET, F., LE ROUX, A., SEVERE, A AND BOEUF, G., 1996. Effects of temperature on growth and metabolism in juvenile turbot. Journal of Fish Biology, **49:** 678-692.

CANAGARATNAM, P., 1966. Observation of feed efficiency and conversion and overall growth of Nile Tilapia, *Oreochromis niloticus* in varied salinities. Asian Journal of Agricultural Science, **4:** 102-123.

CATACUTAN, M. R AND COLOSO, R. M., 1995. Effect of dietary protein to energy rations on growth, survival, and body composition of juvenile Asian seabass,(*Lates calcarifer)*. Aquaculture **13:** 125 -133.

CHAVEZ, M. O. P., LE ROUZIC, C., CAUTY, M., EDERY, T AND PRUNET, M., 1983. Expression of the prolactin receptor (t1PRL-R) gene in tilapia *Oreochromis niloticus*, tissue distribution and cellular localization in Osmoregulatory organs. Journal of Molecular Endocrinology, **24(2):** 215-224.

CLAIREAUX, G AND LAGARDERE, J. P., 1999. Influence of temperature, oxygen and salinity on the metabolism of the European sea bass. Journal of Sea Research, **42:** 157-168.

COOKE, S. J., CHANDROO, K. P., BEDDOW, T. A., MOCCIA, R. D AND MCKINLEY, R. S., 2000. Swimming activity and energetic expenditure of captive rainbow trout, *Oncorhynchus mykiss* estimated by electromyogram telemetry. Aquaculture, **31:** 495-510.

CRUZ, Y AND LAUDENCCIA, M., 1978. Respiromety methods and feed consumption for fish biology. American Journal of Fisheries Society**, 83:** 335-362.

DAS, M. M. A., ISLAM, B AND MUGHAL, G. U., 1992. Induced breeding and fry rearing of *Clarias batrachus*. Bangladesh Journal of Zoology**, 20:** 87-95.

DEACON, N., 1997. Determination of the optimum environmental requirements of juvenile marine fish. The development of a protocol. Ph.D. Thesis, Rhodes University, Grahamstown, South Africa **42:** 157-168.

DENDRINOS, P AND THORPE, J. P., 1985. Effects of reduced salinity on growth and body composition in the European bass *Dicentrarchus labrax*. Aquaculture, **49:** 333-358.

DOF, DEPARTMENT OF FISHERIES., 2011-2012. Fisheries Statistical Yearbook of Bangladesh, Fisheries Resources Survey System (Revised Edition), **27:** 40-43.

DOOLGINDACHABAPORN, L., 1994. Physiological responses during stress and subsequent recovery at different salinities in adult pejerrey *Odontesthes bonariensis.* Aquaculture, **200:** 349–362.

DUTIL, J. D., LAMBERT, Y AND BOUCHER, E., 1997. Does higher growth rate in Atlantic cod (*Gadus morhua*) at low salinity result from lower standard metabolic rate or increased protein digestibility? Canadian Journal of Fisheries and Aquatic Sciences, **54:** 99-103.

DUTIL, J. D., MUNRO, J., AUDET, C AND BESNER, M., 1992. Seasonal variation in the physiological response of Atantic cod (*Gadus morhua*) to low salinity. Canadian Journal of Fisheries and Aquatic Sciences, **49:** 1149-1156.

FEBRY, R AND LUTZ, P., 1987. Mechanisms of hatching and measuring fecundity in fish. Academic Press, London, **3:** 546-550pp.

FERRARIS, R. P., CATACUTAN, M. R., MABELIN, R. L AND ADAN, P.J., 1986. Digestibility in milkfish, *Chanos chanos*, effects of protein source, fish size and salinity. Aquaculture, **59:** 93-105.

FIELDER, T AND BARDSLEY, J., 1999. Utilization of carbohydrate and lipid as dietary energy sources by stinging catfish, (*Heteropneustes fossilis*). Proceedings of the Fourth Asian Fish Nutrition Workshop. Asian Fisheries Society, Manila, Philippines**, 4:** 93-l00.

FOSS, T., BRICK, M. E AND CECH, J. J., 2001. Metabolic responses of juvenile striped bass to exercise and handling stress with various recovery environments. Transactions of the American Fisheries Society, vol.**131:** 855-864.

FRACALOSSI, K AND LOVELL, H., 1995. The effect of reduced salinity on growth, food conversion and protein efficiency ratio in juvenile spotted grunter, *Pommadasys commersonnii*. Aquaculture**, 30:** 13-20.

FRANCOIS, N. R., LAMARRE, S. G AND BLIER, P. U., 2004. Tolerance, growth and haloplasticity of the Atlantic wolfish (*Anarhichas lupus*) exposed to various salinities. Aquaculture, **236:** 659-675.

FRICK, N.T AND WRIGHT, P. A., 2002. Nitrogen metabolism and excretion in the mangrove killifish *Rivulus marmoratus* and the influence of environmental salinity and external ammonia. Journal of Experimental Biology, **205:** 79-89.

GAUMET, F., BOEUF, G., SEVERE, A., LE ROUX, A AND MAYER-GOSTAN, N., 1995. Effects of salinity on the ion balance and growth of juvenile turbot. Journal of Fish Biology, **47:** 865-876.

GHEYAS, J., 1998. Studies on the young grey mullet, Mugil cephalus on effect of salinity on food intake, growth and food conversion. Aquaculture**, 7:** 327- 338.

GORDON, M. S., 1959. Ionic regulation and determination of specific growth rate in the Brown trout (*Salmo trutta*). Journal of Experimental Biology, **36:** 227-252.

GUERIN, J. L AND STICKLE, W. B., 1997. Effect of salinity on survival and bioenergetics of juvenile lesser blue crabs, *Callinectes similes*. Marine Biology,vol. **129:** 63-69.

HAMILTON, G. S., 1822. Water quality criteria and nomenclature for freshwater fish. 2nd ed. Food and Agriculture Organization of the United Nations, FAO, Rome**, 7:** 6-9.

HANDELAND, A., BOULOS, Z AND TERMAN, M., 2000. Food availability and daily biological rhythms. Neuroscience and Biobehavioral Reviews**,**vol. **4:** 119-131.

HASAN, M. R., HAEUE, A. K. M., ISLAM, M.A AND KHAN, E. U. K., 1982. Studies on the effect of stocking density on the growth of Blue tilapia (*Sarotherodon nilotica*) in floating ponds. Bangladesh Journal of Fisheries Research, **25**(1): 73-81.

HASAN, M. R., HAEUE, A. K. M., LSLAM, M. A AND TAREQUE, A. M. M., 1998. Studies on the effects of supplemental feed on the growth of Nile tilapia in floating ponds. Bangladesh Journal of Fisheries Research**, l2** (l): 37-41.

HILE, K. J., 1936. Systematic sources of bias in a bioenergetics model: examples for age-0 striped bass. Transactions of the American Fisheries Society, **122:** 912–926.

HOAR, W. S AND RANDAL, D. J., 1969. Growth and survival of the Atlantic wolfish (*Anarhichas lupus*) exposed to various salinities. American Journal of Fish Biology, **32:** 15-30.

HOGENDOORN, W., CONIDES, A. J., PARPOURA, A. R AND FOTIS, G., 1981. Study of the effects of salinity on the fry of the euryhaline species gilthead sea bream (*Sparus sparus*). Journal of Aquaculture in the Tropics, **12:** 297-303.

HOLLIDAY, F. G. T AND JONES, M. P., 1967. Some effects of salinity on the development of eggs and larvae of the Plaice (*Pleuronectes platessa*). The Asian Journal of Agricultural Science**, 47:** 39-48.

HOPKIN, K. D. M., RIDHA, D., LECLERCA, A. A., AL-AMEERI, S AND AL-AHMED, B., 1989. Screening tilapia for culture in seawater in Kuwait. Aquaculture, **20:** 9-17.

HOPKINS, S. W., 1992. Consumption, growth and allometry two carp species (*Cyrinus carpio, Cirhinnus mrigala*) in different salinities. Canadian Journal of Fisheries and Aquatic Sciences **48:** 1334–1337.

IMSLAND, A. K., FOSS, A., GUNNARSSON, S., BERNTSSEN, M. H. G., GERALD, R. F., BONGA, S. W., VAN HAM, E., NAEVDAL, G AND STEFANSSON, S. O., 2001. The interaction of temperature and salinity on growth and food conversion in juvenile turbot (*Scophthalmus maximus*). Aquaculture, **198:** 353-367.

ISLAM, A. K. M. N AND SAHA, S. K., 1975. Limnological studies of the Ramna Lake at Dhaka.mBangladesh Journal of Fisheries Research, **23:**39-48.

ISLAM, M. A. S. M., RAHMATULLAH, A AND SALAM, M. A., 1996. Effect of food and stoking density on the rearing of shingi *Heteropneustes* fossilis fry. Asian Journal of Agricultural Science**. 25:** 73-78.

ISMLAND, A. K., FOLKVORD, A AND STEFANSSON, S. O., 1995. Growth, oxygen consumption and activity of juvenile turbot, *Scophthalmus maximus* reared under different temperatures and photoperiods. Journal of Sea Research, **34:** 149-159.

JINGRAN, V. G., 1982. Growth performance and feeding behavior of juvenile Nile Tilapia (*Oreochromis niloticus*). Fish and Fisheries of India,vol. **48:** 1334–1337.

JOB, S. U., 1969. A synopsis of ecological information in the saline lake developments and hydrobiology of rotifer *Brachionus plicatilis*. Mexican Journal of Agriculture, **20:** 156– 162.

JONES, A AND RANDALL, J., 1978. Growth rates of carp fishes in different salinities. Canadian Journal of the Fisheries Research, **16:** 121-130.

JONES, W. T., 1967. Growth and feeding of juvenile cod (*Gadus morhua*) in different salinities. Journal European Science**, 42:** 11–32.

KASELOO, P. A., WEATHERLY, A. H., LOTIMER, J AND FARINA M. D., 1992. A biotelemetry system recording in fish activity. Journal of Fish Biology, **40:** 165-179.

KASIM, H. M., 1983. Salinity tolerance of certain freshwater fishes. Indian Journal of Fisheries**, 30:** 46–54.

KHAN, S AND SARKAR, M. R., 2004. Improvement in pangas as (*pangasius hypophthalmas*) farming by using silver carp (*Hypophthalmichthys molitrixs*) to reduce the effect of eutrophication. Bangladesh Journal of Fisheries Research, **27:**50-53.

KIBRIA, G. D., NUGEGADA, P. L AND FAIRCHLOUGH, R., 1997. Preliminary rearing Trials of an Australian Native Fish Silver perch (*Bidyanum bictynus*) with reference to growth and production of solid waste in aquaculture. The Journal of Asian Fisheries Society**, 9:** 301- 309.

KILAMBI, R.V AND ZDINAK, A., 1980. The effects of acclimation on the salinity tolerance of carp, (*Ctenopharyngodon idella*). Journal of Fish Biology**, 17:** 71–175.

KOHINOOR, A. H. M., ISLAM, M. L., WAHAB, M. A AND THILSTED, S. H., 1998. Effect of mola (*Amblypharyngodon mola*) on the growth and production of carps in polyculture. Journal of Indian Fisheries Research**, 2**(2): l19-126.

KURATA, H., 1959. Preliminary report on the rearing of herring larvae in lake. Mexican Journal of Agricultural Science**, 20:** 117-138.

LAMBERT, Y., DUTIL, J. D AND MUNRO, J., 1994. Effects of intermediate and low salinity conditions on growth rate and food conversion of Atlantic cod (*Gadus morhua*). Canadian Journal of Fisheries Sciences, **51:** 1569-1575.

LEMARIE, G., BAROILLER, J. F., CLOTA, F., LAZARD, J AND DOSDAT, A., 2004. A simple test to estimate the salinity resistance of fish with specific application to *Oreochromis niloticus and Sarotherodon melanotheron.* Aquaculture**, 24:** 575-587.

LILONGWE, J.S., STECKO, T. D., STAUFFER, J.R AND CARLINE, R. F., 1996. Combined effects of water temperature and salinity on growth and feed utilization of juvenile Nile Tilapia, *Oreochromis niloticus*. Journal of African Science, **146:** 37-46.

LUCAS, M.C., JOHNSTONE, A. D. F AND PRIEDE, I. G., 1993. Use of physiological telemetry as a method of estimating metabolism of fish in the natural environment. Transactions of the American Fisheries Society, **122:** 822-833.

MANDAL, N., 1998. An electronic controller for the automatic determination of oxygen consumption and stability of other water quality parameter. Aquaculture**, 36:** 73-77.

MARTINEZ-PALACIOS, C. A., BARRIGA TOVAR, E., TAYLOR, J. F., RI´OS DURA´N, G., ROSS, L.G., 2004. Effect of temperature on growth and survival of *Chirostoma estor estor* using a simple video technique for remote measurement of length and mass of larval and juvenile fishes. Aquaculture **209:** 369–377.

MARTINEZ-PALACIOS, C. A., BARRIGA TOVAR, E., TAYLOR, J.F., RI´OS DURA´N, G., ROSS, L. G., 2008. Osmoregulatory adjustments by three Atherinids (*Leptatherina presbyteroides, Craterocephalus mugiloides*, *Leptatherina wallace*) to a range of salinities. Journal of Comparatives Biochemistry, **103**(4): 725– 728.

MARTINEZ-PALACIOS, T AND ROSS, L. G., 1986. The ontogeny of salinity tolerance in the tilapias *Oreochronus aureus, Oreochronus niloticus* and an *Oreochronus mossambicus*. Journal of African Science, **47**(4): 353-367.

MCGEACHIN, R. B. R. I., WICKLUND, B. L AND WINTON, J. R., 1987. Growth of *Tilapia aurea* in seawater cages. Journal of World Aquaculture Society, **18:** 31-34.

MCINERNEY, J. E., 1964. Salinity preference, an orientation mechanism in salmon migration, juvenile Salmon Migration Mapping. Canadian Journal of Fisheries Sciences, **31:** 1069-1075.

MEDAWARS, L., 1945. Physiology of salinity tolerance in tilapia an update of basic and applied aspects. Journal of Aquatic Living Resources, **2:** 91– 97.

MILES, H. M AND SMITH, L. S., 1968. Ionic regulation in migrating juvenile Coho salmon, *Oncorhynchus kisutch.* Journal of Sea Research, **26:** 281-398.

MILLER, R. R., 1976. Some effects of seawater and temperature on the embryo of Pacific salmon, *Oncorhynchus gorbuscha and Oncorhynchus keta.* Mexican Journal of Agriculture**, 6:** 79-90.

MINOT, J. F., 1990. Developmental rates of *Menidia audens* with notes on salt tolerance. Transactions of the American Fisheries Society, **100:** 603– 610.

MOLLAH, M. F. A AND HAQUE, A. K. M. A., 1978. Studies on monthly variations of plankton in relation to the physico-chemical condition of water a bottom soil of two ponds Zooplankton. Bangladesh Journal of Fisheries Resource**, l2:** 99-103.

MORGAN, H AND IWAMA, M., 1991. A mass mortality of fish associated with low salinity in the Bot River estuary. Transactions of the Royal Society of South Africa, **45:** 437-447

MUIR, C AND ROBERTS, J., 1995. Metabolic cost of acute physical stress in juvenile steelhead. Transactions of the American Fisheries Society, **116:** 257-263.

MUMTAZUDDIN, M. M. S., RAHMAN, M AND MOSTAFA, G., 1982. Limnological studies of four selected ponds at the Aquaculture Ext. Station, Mymensingh. Bangladesh Journal of Fisheries Resource**, 5:** 83-90.

MURTY, L. A AND EVANS, D. H., 1978. The roles of gill permeability and transport mechanism in euryhalinity. Journal of Fish Physiology**, 4:** 239-283.

NELSON, S. M., 1987. Evaluation of saline spring water for aquaculture. Journal of Aquatic Resources, **27:** 211–216.

NIKOLSKY, G. V., 1963. The ecology and behavioral response of Fishes in different salinities. Academic Press, London, **117:** 258-263pp.

NORDLIE, F. G AND HANEY, D. C., 1998. Adaptations in salt marsh teleosts to life in waters of varying salinity. Italian Journal of Zoology, **65:** 405-409.

NORDLIE, F. G., 1985. Osmotic regulation in the sheep head minnow, *Cyprinodon variegates*. Journal of Fish Biology**, 26:** 161-170.

NORDLIE, F. G., WALSH, S. J., HANEY, D. C AND NORDLIE, T. F., 1991. The influence of ambient salinity on routine metabolism in the teleost *Cyprinodon variegates*. Journal of Fish Biology, **38:** 115-122.

NUGON, R. W., 2003. Salinity tolerance of juveniles of four varieties of tilapia. M.Sc. Thesis, Louisiana State University, USA, **36:** 173-177.

OPPENHEIMER, J. R., AFIMED, M. G., HUQ, A., HAQUE, K. A., ALAM, A. K. M.A., AZIZ, K. M., ALI, S AND HAQUE, A.S.M.N., 1978. Limnological studies of three ponds in Dhaka, Bangladesh. Bangladesh Journal of Fisheries Resource, **l:** 1-8.

OTTO, W. T., 1971. The role of sense organs in the feeding behavior of juvenile sole and plaice. Journal of Fish Biology, **47:** 931-939.

PARRY, G., 1966. Osmotic adaptation and specific growth in fish in different salinities. Italian Journal of Zoology, **51:** 292–344.

PAYNE, C. T., 1987. A generalized bioenergetics model of fish growth, measuring in Microcomputers, version 2.0. Technical Report WIS-SG-92-250, University of Wisconsin, Sea Grant Institute**, 31:** 362–370pp

PETERSON, A., BEAMISH, F. W. H AND MOOKHERIJII, P. S., 1999. Respiration of fishes with special emphasis on standard oxygen consumption and influence of weight and temperature on respiration of goldfish, *Carassius auratus*. Canadian Journal of Zoology, **42:** 161-175.

RAHMAN, M. A., BHADRA, A., BEGUM, N AND HUSSAIN, M. G., 1997. Eflects of some selective supplemental feeds on the survival and growth of catfish (*Clarias batrachus*) fry. Bangladesh Journal of Fisheries Resource**, l** (2): 55-58.

RAHMAN, M. S., CHOWDHYRY, M. Y., FIAQUE, A. K. M. A AND HAQUE, M. S., 1982. Limnological studies and biodiversity assessment for four ponds at Dhaka city, Bangladesh. Bangladesh Journal of Fisheries Resource, **5**(l): 25-35.

RANKIN, J. C AND BOLIS, L., 1984. Hormonal control of water movement across the gills of carp fishes. Journal of Fish Physiology, **5:** 177-201.

RESENDEZ, M. A., 1981. Preliminary observation on salinity tolerance of three sizes of the GIFT and Nonimproved strains of the Nile Tilapia, *Oreochromis niloticus*. European Journal of Science, **24**(3): 373-377.

RESLEY, M. J., WEBB J, K. A AND HOLT, G. J., 2006. Growth and survival of juvenile cobia, *Rachycentron canadum* at different salinities in a recirculating aquaculture system. Aquaculture, **253:** 898-907.

RUBIA, S., AL ABDUL ELAH, K. M AND TENG, S., 2005. Effects of salinity and water temperature on the survival and growth of brown spotted grouper (*Epinephelus tauvina*) larvae. Journal of the World Mariculture Society, **14:** 624-635.

SAILLANT, A AND BENNETT, B. A., 2003. Long term trends in the catches by shore anglers in Bay. Transactions of the Royal Society of South Africa**, 47:** 683-690.

SAUNDERS, R. L AND HENDERSON, E. B., 1970. Influence of photoperiod on development and growth in Atlantic salmon (*Salmo salar*). Journal of the Fisheries Research Board of Canada **4:** 1295-12311.

SECOR, B., BULLIVAN, J. S AND SHAW, T., 2000. The influence of salinity on the rate of oxygen consumption of young quinnat salmon (*Onchorhynchus tshawytscha*). New Zealand Journal of Science**, 4:** 381-391.

SHAW, T., AKBER, M AND ABUD, E. O. A., 1975. Effects of salinity and weight on routine metabolism in the juvenile croaker, *Micropogonias furnieri*. Journal of Fish Biology, **40:** 471- 472.

SPARKS, D., BLACKBURN, J AND CLARKE, W. C., 2003. Revised procedure for the 24 hour challenge test to measure seawater adaptability of juvenile salmonids. Canadian Technical Report of Fisheries and Aquatic Sciences, **32:** 1515-1535.

SRDI, SOIL RESOURCE DEVELOPMENT INSTITUTE., 1998. A comparison of salinity regimes in nearby permanently open, seasonally open and normally closed estuaries in the South Asia. South Asian Journal of Marine Sciences, **8:** 43-55.

SUN, L., CHEN, H., HUANG, L., WANG, Z AND YAN, Y., 2006. Growth and energy budget of juvenile cobia (*Rachycentron canadum*) relative to ration. Aquaculture, **257:** 214-220.

SWANSON, C., 1998. Interactive effects of salinity on metabolic rate, activity, growth and osmoregulation in the euryhaline milkfish (*Chanos chanos*). Journal of Experimental Biology, **201:** 330-336

SWINGLE, H. S., 1957. Relationships of pH of pond water of their suitability for fish culture. New Zealand Journal of Science**, 3:** 281-291.

TANDLER, A. M., HASAN, M. R AND HABIB, M. A. B., 1995.Observing feeding response and utilization of carbohydrate and lipid as dietary energy sources by stinging catfish, (*Heteropneustes fossilis*). Proceedings of the 2nd Asian Fish Nutrition Workshop. Asian Fisheries Society, Manila, Philippines, **19:** 83-l10.

TARER, S. R., 2000. The effect of salinity on the growth and tolerance of *Labeo rohita* and *Cirrhinus mrigala*, M. Phil. Thesis, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan**, 7:** 31-39.

VARSAMOS, S., WENDELAAR BONGA, S. E., CHARMANTIER, G AND FLIK, G., 2004. Drinking and Na+/K+ ATPase activity during early development of European sea bass, *Dicentrarchus labrax*, ontogeny and short-term regulation following acute salinity changes. Journal of Experimental Marine Biology and Ecology, **311:** 189-200.

WEDEMEYER, L., 2001. The ontogeny of salinity tolerance in the tilapias *Oreochromis aureus*, *Oreochromis niloticus* and *Oreochromis mossambicus, Oreochromis niloticus* hybrid, spawned and reared in freshwater. Aquaculture **47:** 353– 367.

WOO, R. T AND KELLY, M., 1995. Environmental factors influencing growth of fish. Journal of Fish Physiology**, 78:** 599-675.

WOO, R. T AND WU, M., 1982. The ichthyofauna of the Sundays Estuary, South Africa, with particular reference to the juvenile marine component. Journal of African Science **7:** 248-258.

ZEITOUN, I. H., KUO, C. M AND HUANG, M. C., 1973. The ontogeny and growth performance of salinity tolerance in the tilapias *Oreochromis aureus, Oreochromis niloticus* and *Oreochromis mossambicus*. Asian Journal of Agricultural Science **48:** 153– 167.

Appendices

APPENDIX 01

1 (a)

SGR

	TREATMENT	N	Subset for alpha = .05			
			1	2	3	4
Tukey HSD(a)	15 ppt 30	2	.075			
	15 ppt 15	2	.075			
	12 ppt 45	2	.080			
	15 ppt 45	2	.080			
	12 ppt 30	2	.085	.085		
	15 ppt 60	2	.090	.090		
	12 ppt 60	2	.110	.110		
	12 ppt 15	2	.125	.125		
	9 ppt 15	2	.175	.175		
	9 ppt 30	2	.175	.175		
	9 ppt 45	2		.185		
	9 ppt 60	2		.185		
	6 ppt 45	2			.935	
	6 ppt 30	2			.945	
	3 ppt 45	2			.960	
	6 ppt 15	2			.960	
	6 ppt 60	2			.960	
	3 ppt 15	2			.975	
	3 ppt 60	2			.990	
	3 ppt 30	2			.995	
	0 ppt 30	2				1.140
	0 ppt 45	2				1.140
	0 ppt 15	2				1.155
	0 ppt 60	2				1.165
	Sig.		.074	.074	.767	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 2.000.

1 (b)

FCR

	TREATMENT	N	Subset for alpha = .05				
			1	2	3	4	5
Tukey HSD(a)	0 ppt 60	2	1.550				
	0 ppt 15	2	1.750				
	3 ppt 30	2	1.850				
	0 ppt 45	2	1.900	1.900			
	3 ppt 60	2	1.900	1.900			
	0 ppt 30	2	2.000	2.000	2.000		
	6 ppt 60	2	2.050	2.050	2.050		
	3 ppt 15	2	2.300	2.300	2.300		
	6 ppt 15	2	2.350	2.350	2.350		
	6 ppt 30	2	2.400	2.400	2.400		
	3 ppt 45	2		2.750	2.750		
	6 ppt 45	2			2.800		
	9 ppt 45	2				4.100	
	9 ppt 30	2				4.150	
	9 ppt 60	2				4.200	
	9 ppt 15	2				4.350	4.350
	12 ppt 45	2				4.600	4.600
	12 ppt 30	2				4.700	4.700
	12 ppt 60	2				4.700	4.700
	12 ppt 15	2				4.800	4.800
	15 ppt 30	2				4.800	4.800
	15 ppt 15	2				4.850	4.850
	15 ppt 60	2				4.950	4.950
	15 ppt 45	2					5.200
	Sig.		.080	.080	.125	.080	.080

Means for groups in homogeneous subsets are displayed.

a Uses Harmonic Mean Sample Size = 2.0

1 (c)

AGR

	TREATMENT	N	Subset for alpha = .05					
			1	2	3	4	5	6
Tukey HSD(a)	15 ppt 60	2	.010					
	15 ppt 45	2	.015					
	12 ppt 15	2	.025	.025				
	15 ppt 30	2	.025	.025				
	12 ppt 30	2	.030	.030				
	12 ppt 60	2	.030	.030				
	15 ppt 15	2	.030	.030				
	9 ppt 30	2	.035	.035	.035			
	9 ppt 45	2	.035	.035	.035			
	12 ppt 45	2	.035	.035	.035			
	9 ppt 60	2	.040	.040	.040	.040		
	9 ppt 15	2	.050	.050	.050	.050	.050	
	6 ppt 45	2		.090	.090	.090	.090	.090
	6 ppt 15	2		.095	.095	.095	.095	.095
	6 ppt 30	2		.095	.095	.095	.095	.095
	3 ppt 45	2			.105	.105	.105	.105
	3 ppt 30	2			.105	.105	.105	.105
	3 ppt 15	2				.110	.110	.110
	0 ppt 45	2					.115	.115
	6 ppt 60	2					.120	.120

3 ppt 60	2					.120	.120
0 ppt 30	2						.125
0 ppt 15	2						.135
0 ppt 60	2						.145
Sig.		.804	.064	.064	.064	.064	.308

Means for groups in homogeneous subsets are displayed

a Uses Harmonic Mean Sample Size = 2.000.

1 (d)

SURVIVAL

	TREATMEN	N	Subset for alpha = .05					
			1	2	3	4	5	6
Tukey HSD(a)	15 ppt 60	2	20.000					
	15 ppt 45	2	40.000	40.000				
	15 ppt 30	2		55.000	55.000			
	12 ppt 60	2		60.000	60.000	60.000		
	12 ppt 45	2			67.500	67.500	67.500	
	15 ppt 15	2			75.000	75.000	75.000	
	12 ppt 30	2				80.000	80.000	80.000
	12 ppt 15	2					85.000	85.000
	0 ppt 15	2						100.000
	0 ppt 30	2						100.000
	0 ppt 45	2						100.000
	0 ppt 60	2						100.000
	3 ppt 15	2						100.000
	3 ppt 30	2						100.000
	3 ppt 45	2						100.000
	3 ppt 60	2						100.000
	6 ppt 15	2						100.000
	6 ppt 30	2						100.000
	6 ppt 45	2						100.000
	6 ppt 60	2						100.000
	9 ppt 15	2						100.000

9 ppt 30	2						100.000
9 ppt 45	2						100.000
9 ppt 60	2						100.000
Sig.		.064	.064	.064	.064	.169	.064

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 2.000.

APPENDIX 02

2 (a)

AGR

				FIFTEEN	THIRTY	FRFIVE	SIXTY
TREATMEN	0 ppt	1		.1	.11	.08	.15
		2		.1	.14	.15	.14
		Total	N	2	2	2	2
			Mean	.135	.1250	.1150	.1450
			Std. Error of Mean	.0050	.01500	.03500	.00500
	3 ppt	1		.1	.10	.12	.14
		2		.1	.11	.09	.10
		Total	N	2	2	2	2
			Mean	.110	.1050	.1050	.1200
			Std. Error of Mean	.0100	.00500	.01500	.02000
	6 ppt	1		.1	.09	.08	.11
		2		.1	.10	.10	.13
		Total	N	2	2	2	2
			Mean	.095	.0950	.0900	.1200
			Std. Error of Mean	.0150	.00500	.01000	.01000
	9 ppt	1		.0	.03	.02	.03

	2		.1	.04	.05	.05
	Total	N	2	2	2	2
		Mean	.050	.0350	.0350	.0400
		Std. Error of Mean	.0100	.00500	.01500	.01000
12 ppt	1		.0	.03	.05	.04
	2		.0	.03	.01	.01
	Total	N	2	2	2	2
		Mean	.025	.0300	.0300	.0250
		Std. Error of Mean	.0150	.00000	.02000	.01500
15 ppt	1		.0	.03	.01	.01
	2		.0	.02	.02	.01
	Total	N	2	2	2	2
		Mean	.030	.0250	.0150	.0100
		Std. Error of Mean	.0000	.00500	.00500	.00000
Total	N		12	12	12	12
	Mean		.074	.0692	.0650	.0767
	Std. Error of Mean		.0129	.01234	.01323	.01639

a Limited to first 100 cases.

2 (b)

SGR

				FIFTEEN	THIRTY	FRFIVE	SIXTY
TREATMEN	0 ppt	1		1.1	1.12	1.09	1.17
		2		1.2	1.16	1.19	1.16
		Total	N	2	2	2	2
			Mean	1.155	1.1400	1.1400	1.1650
			Std. Error of Mean	.0150	.02000	.05000	.00500
	3 ppt	1		1.0	1.01	.98	.99
		2		1.0	.98	.94	.99
		Total	N	2	2	2	2
			Mean	.975	.9950	.9600	.9900
			Std. Error of Mean	.0050	.01500	.02000	.00000
	6 ppt	1		1.0	.98	.95	.97
		2		.9	.91	.92	.95
		Total	N	2	2	2	2
			Mean	.960	.9450	.9350	.9600
			Std. Error of Mean	.0200	.03500	.01500	.01000
	9 ppt	1		.2	.19	.19	.20
		2		.2	.16	.18	.17

	Total	N	2	2	2	2
		Mean	.175	.1750	.1850	.1850
		Std. Error of Mean	.0050	.01500	.00500	.01500
12 ppt	1		.1	.10	.09	.12
	2		.1	.07	.07	.10
	Total	N	2	2	2	2
		Mean	.125	.0850	.0800	.1100
		Std. Error of Mean	.0050	.01500	.01000	.01000
15 ppt	1		.1	.11	.08	.09
	2		.1	.04	.08	.09
	Total	N	2	2	2	2
		Mean	.075	.0750	.0800	.0900
		Std. Error of Mean	.0050	.03500	.00000	.00000
Total	N		12	12	12	12
	Mean		.577	.5692	.5633	.5833
	Std. Error of Mean		.1380	.13959	.13716	.13883

a Limited to first 100 cases.

2 (c)

FCR

				FIFTEEN	THIRTY	FRFIVE	SIXTY
TREATMEN	0 ppt	1		1.8	2.10	2.10	1.60
		2		1.7	1.90	1.17	1.50
		Total	N	2	2	2	2
			Mean	1.750	2.0000	1.6350	1.5500
			Std. Error of Mean	.0500	.10000	.46500	.05000
	3 ppt	1		2.2	1.90	2.40	1.90
		2		2.4	1.80	3.10	1.90
		Total	N	2	2	2	2
			Mean	2.300	1.8500	2.7500	1.9000
			Std. Error of Mean	.1000	.05000	.35000	.00000
	6 ppt	1		2.3	2.60	2.90	2.20
		2		2.4	2.20	2.70	1.90
		Total	N	2	2	2	2
			Mean	2.350	2.4000	2.8000	2.0500
			Std. Error of Mean	.0500	.20000	.10000	.15000
	9 ppt	1		4.3	3.80	4.00	4.30
		2		4.4	4.50	4.20	4.10

	Total	N	2	2	2	2
		Mean	4.350	4.1500	4.1000	4.2000
		Std. Error of Mean	.0500	.35000	.10000	.10000
12 ppt	1		4.7	4.60	4.60	4.50
	2		4.9	4.80	4.60	4.90
	Total	N	2	2	2	2
		Mean	4.800	4.7000	4.6000	4.7000
		Std. Error of Mean	.1000	.10000	.00000	.20000
15 ppt	1		4.8	4.70	5.00	4.70
	2		4.9	4.90	5.40	5.20
	Total	N	2	2	2	2
		Mean	4.850	4.8000	5.2000	4.9500
		Std. Error of Mean	.0500	.10000	.20000	.25000
Total	N		12	12	12	12
	Mean		3.400	3.3167	3.5142	3.2250
	Std. Error of Mean		.3898	.38393	.37716	.42961

a Limited to first 100 cases.

2 (d)

SURVIVAL

				FIFTEEN	THIRTY	FRFIVE	SIXTY
TREATMEN	0 ppt	1		100.0	100.00	100.00	100.00
		2		100.0	100.00	100.00	100.00
		Total	N	2	2	2	2
			Mean	100.000	100.0000	100.0000	100.0000
			Std. Error of Mean	.0000	.00000	.00000	.00000
	3 ppt	1		100.0	100.00	100.00	100.00
		2		100.0	100.00	100.00	100.00
		Total	N	2	2	2	2
			Mean	100.000	100.0000	100.0000	100.0000
			Std. Error of Mean	.0000	.00000	.00000	.00000
	6 ppt	1		100.0	100.00	100.00	100.00
		2		100.0	100.00	100.00	100.00
		Total	N	2	2	2	2
			Mean	100.000	100.0000	100.0000	100.0000
			Std. Error of	.0000	.00000	.00000	.00000

		Mean				
9 ppt	1		100.0	100.00	100.00	100.00
	2		100.0	100.00	100.00	100.00
	Total	N	2	2	2	2
		Mean	100.000	100.0000	100.000 0	100.000 0
		Std. Error of Mean	.0000	.00000	.00000	.00000
12 ppt	1		90.0	80.00	70.00	70.00
	2		80.0	80.00	65.00	50.00
	Total	N	2	2	2	2
		Mean	85.000	80.0000	67.5000	60.0000
		Std. Error of Mean	5.0000	.00000	2.50000	10.0000 0
15 ppt	1		80.0	60.00	50.00	25.00
	2		70.0	50.00	30.00	15.00
	Total	N	2	2	2	2
		Mean	75.000	55.0000	40.0000	20.0000
		Std. Error of Mean	5.0000	5.00000	10.0000 0	5.00000
Total	N		12	12	12	12
	Mean		93.333	89.1667	84.5833	80.0000
	Std. Error of Mean		3.0977	5.14315	7.11002	9.31356

a Limited to first 100 cases.