

**Development of Nursery Feed for *Heteropneustes fossilis* with
Particular Reference to Salinity Stress Tolerance**



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Particular Reference to Salinity Stress Tolerance**

A Thesis

By

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**Development of Nursery Feed for *Heteropneustes fossilis* with
Particular Reference to Salinity Stress Tolerance**

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Declaration

Development of Nursery Feed for *Heteropneustes fossilis* with Particular Reference to Salinity Stress Tolerance

The results submitted in this thesis are entirely of the candidate's own investigations and no part of the results has been accepted for any degree, nor is it being concurrently submitted for any degree.

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April, 2014

Dedicated to

Our Freedom Fighters

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Mirja Kaizer Ahmmed

Abstract

The present study was conducted to assess the suitability of different larval feeds of *Heteropneustes fossilis* with particular reference to salinity stress tolerance. For this purpose, 30 days old *Heteropneustes fossilis* larvae were collected from local hatchery at Cachra in Jessore. The feeding trial was conducted in triplicate with 30 larvae (0.74 ± 0.01 g; 2.95 ± 0.12 cm) in glass aquaria ($54 \times 36 \times 36$ cm³) each containing 30L water. Four different feeding treatments *i.e.* *Tubifex* spp. (T₁), commercial pellet feed (T₂), snail meat (T₃) and mixed diet *i.e.* commercial feed and snail meat on alternative day (T₄) were applied. The larvae containing each treatment were fed two times per day with that particular feed until reaching satiation level. The larvae fed with tubificid worms (T₁) and mixed diet (T₄) showed significantly better results ($P < 0.05$) in terms of length and weight gain, percent weight gain and specific growth rate (SGR %) compared to the rest two treatments. Water quality parameters were monitored throughout the experimental periods and the parameters did not vary significantly ($P > 0.05$) among the treatments. On the basis of larval development and survival rate, it could be suggested that the live tubificid worms (T₁) and mixed diet (T₄) are suitable for *Heteropneustes fossilis* larvae.

For determining particular salinity stress tolerance, there were six treatments, each having three replications, stocked with 30 larvae (4.4 ± 0.3 cm; 1.59 ± 0.11 g) in glass aquarium ($54 \times 36 \times 36$ cm³). The larvae were cultured in aquarium with five different salinities 5, 8, 10, 12 and 15 ppt for 30 days to determine the optimum salinity level for direct stocking. The larvae were fed with the best feed (commercial feed and snail meat on alternative day) from the previous experiment. The larvae were exposed to different salinity directly from fresh water. The result reveals that, in case of direct stocking 10ppt, 12ppt and 15ppt are lethal salinity for *Heteropneustes fossilis* larvae. About 70% survivability was recorded in 8ppt salinity level that is quite satisfactory. But the larvae stocked at 8ppt showed comparatively poor growth rather than 0ppt and 5ppt. In the next part of the experiment, salinity was increased gradually 1ppt in every 3 days. The result revealed 100% survivability up to 7ppt. The survivability rate was also satisfactory at 8ppt (98%), 9ppt (97%) and 10ppt (76%) salinity. The salinity level more than 13ppt can be marked as lethal salinity level for *Heteropneustes fossilis* larvae. So, the present study suggests that the *Heteropneustes fossilis* larvae can be stocked directly at 5ppt salinity and can be cultured in 0 to 10ppt salinity level.

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Chapter 1

Introduction

1. Introduction

Bangladesh is an agro-based riverine country enriched with enormous fisheries resources. Fisheries sector plays an important role in the socio-economic development, nutrition supply, employment generation, poverty alleviation and foreign exchange earning of Bangladesh. During the last three decades a great attention was paid towards aquaculture as an attempt to fulfill the gap between the increased population and their demand for animal protein. Unfortunately, fish production from open water capture fisheries has gradually declined during 1985 to 1996 due to modification and reduction of fish habitats, loss of fish breeding and nursery grounds, blocking of migratory channels by construction of flood control drainage (FCD) and flood control drainage and irrigation (FCDI) schemes, water pollution and diseases like Epizootic Ulcerative Syndromes (EUS) etc (Shahin *et al.*, 2011). Gradual decline of inland capture fisheries, aquaculture has evolved to play an important role in the total fish production in this country. It has become the fastest growing sector of food production in the world.

In Bangladesh, both fresh water and brackish water aquaculture are practiced in the coastal area. The freshwater area is getting reduced and the overall ecology of fish habitats and the routes of migration have altered due to various water resources development activities. So, it is very difficult to fulfill the minimum protein requirement of the teeming millions from the freshwater sub-sector alone. But the highest priority has always been accorded to the freshwater fisheries, as reflected in the number of fisheries development projects implemented since liberation, in spite of that the marine and brackish water fisheries sectors have the lion's share of foreign exchange earnings and contributes to the development budget in that proportion. If similar level of management and development attention was paid to the marine and brackish water sectors, it would be possible to give substantially increased production.

The geographical position and climatic condition of Bangladesh have made her coastal areas one of the highly productive areas of the world (Islam, 2003). The coastal and marine fisheries have been playing considerable roles not only in the social and economic development of the country but also in the regional ecological balance. About 20 million people live in the coastal region of Bangladesh; some are

from the poorest and most marginalized groups in the country, 20 percent of them directly depend on the coastal and marine resources for their livelihood.

Shrimp was once much cheaper locally than fish and was not considered an attractive food item. In the early Seventies, however, Bangladesh entered the world export market for shrimp and since then this sector has suddenly become a very high priced-commodity. Farmers cultured shrimp for the fulfillment of the export market demand and they earned good amount of foreign currency. But now a days, diseases caused by biological or non-biological agent is becoming a burning issue for aquaculture industry. In spite of the great potentiality, shrimp culture of Bangladesh is affected with a wide range of microbial disease problem. In most of the cases there are no effective ways of treatment which result poor production and great economic loss. In this case if fishermen get any option to culture some demandable species with will be a good source of minimizing the risk as well as they can earn handsome amount of money.

It is mentionable that, the native species are threatened now due to poorly planned water management policy for irrigation, over exploitation, illegal fishing and various ecological changes in its natural habitat (Chakraborty, 2010). So, it is time to take attention on the culture of this native species considering the importance of these species from the nutritional, economic and biodiversity point of view.

One of the commercially important native fish species in Bangladesh, the Asian stinging cat fish, Shing or scientifically known as *Heteropneustes fossilis* belongs to the family Heteropneustidae can be selected (Rahman et al., 2013). It has both high market value and consumer acceptance. The species has high content of iron (226 mg 100 g⁻¹) and fairly high content of calcium compared to many other freshwater fishes (Saha and Guha, 1939). Due to its high nutritive value the fish is recommended in the diet of the sick and the convalescents (Singh Kohli and Goswami, 1989). Being a lean fish it is very suitable for people for whom animal fats are undesirable (Rahman et al., 1982). Few days ago, seed unavailability was the main problem for culturing native species. But the situation is going to change day by day. Hatcheries producing native fish fry are developing all over the country. Recently, a good number of shing hatchery has been developed at Chachra in Jessore district.

A preliminary survey was conducted to find out the present condition and problems of the native fish hatchery specially Shing hatchery at Chachra. The survey result found about 52 hatcheries were available at Chachra who produced different types of native fish fry. Among them, about 40% hatcheries were producing Shing fry for commercial purposes. There are about 10% hatcheries who were exclusively producing Sing fry for commercial purposes. Number of sing producing hatchery is increasing day by day. People culture brood fish in pond for their hatchery.

All hatchery operators are efficient in breeding techniques of shing. They followed standard breeding procedure of Shing. About 3,50,000 – 4,00,000 Shing fries were produced last year from 20% hatchery of Jessore. These fries were transported to different parts of the country. About 95% hatchery operator's had the facility of oxygenated polythene bag and half dram facilities for transportation. In some cases, normal polythene bag were used for short distance transportation. Load-shedding is a common problem in our country. Every hatchery operators had the alternative electricity facility. To run a culture practice commercially induced breeding, larval rearing, grow out and brood stock maintenance is very essential. Among various stages of life, larval stage is considered one of the most sensitive phases during their life cycle. Based on survey result, induced breeding, larval rearing, grow out and brood stock maintenance are well developed for Shing.

Well balanced and suitable diets are one key biological component for support good growth and health of fish. Survey result found that all of the hatchery owner use tubifex as larval feed, whereas the farmer use commercial feed during culture due to higher rate of tubifex. The larval growth remains good at hatchery due to use of live feed. Due to sudden change of food types in culture pond, the constant growing rate of the larvae is hampered and farmers do not get satisfactory growth.

Growth and survival data are powerful tools for understanding the effects of both live and manufactured commercial diets on first feeding fish larvae. Growth and survival data were therefore used in the present study to evaluate the effects of live food and commercial diets on first feeding of shing (*Heteropneustes fossilis*).

Shing is mainly a fresh water species and found in ponds, ditches, beels, swamps and marshes, but sometimes found in muddy rivers (Jha and Rayamajhi, 2010).



They are very hardy fish and can survive for a few hours out of the water due to the presence of accessory respiratory organs. The air-breathing apparatus of stinging catfish enables it to exist in almost any kind of water (Rahman *et al.*, 2013). Its salinity preferences are not documented. It is very important to check the exact salinity tolerance level that will lead to add new coastal species in our country. That is why, particular salinity stress tolerance level were identified based on growth and survival data in different salinity. The present study was conducted to find out the result of the following objectives.

- To develop nursery feed of *Heteropneustes fossilis* and
- To determine particular salinity stress tolerant level for *Heteropneustes fossilis*.

Chapter 2

Literature Review

2. Review of Literature

There have been considerable studies on the feed selection for different fishes in different stages of life. In the present study efforts have been made of consult a few available literatures on the use of different kind of nursery feed for different types of fish.

Sarowar *et al.* (2010) reported that three different diets *i.e.* chopped silvercarp fry, formulated feed and live tubificid worms – having different protein levels for the rearing of *C. striatus* fry. The fry fed with live tubificid worms showed significantly better results in terms of weight and length gain, percent weight and length gain and specific growth rate (SGR%) compared to the rest two treatments. The highest survival rate ($89.14 \pm 2.16\%$) was also shown by the tubificid worms fed larvae which was also significantly higher than those fed chopped silvercarp fry and formulated feed, respectively.

Mahfuj *et al.* (2012) conducted an experiment for 56-day with a view to observe the effects of different feeds on larval development and survival of 3 day-old ornamental koi carp *Cyprinus carpio* larvae in fiber glass aquaria to develop rearing technique for promoting its aquaculture and for promoting the aquarium business. The highest survival rate $56.66 \pm 2.29\%$ was shown by the chopped tubificid worms fed larvae which was significantly higher than those of crushed pellet feed and mixed diet respectively.

Al-Hafedh (1999) reported that the growth rate of fish increases with increase in the level of dietary protein till the optimum level is reached.

Khan *et al.* (1994) started a experiement on grass carp fish and found that excessive protein level over 35% in diet caused a marked decline in weight gain and fish growth. This might have resulted from extra energy available for growth.

Holt (1993) stated that many species of marine and fresh water fish larvae ingest formulated feeds but the associated poor growth is thought to be due to lack of digestion or assimilation of these feeds. For example, an increase of dry feed component of a mixed dry or live diet caused an increase in the percentage of

malformed European minnow larvae, *Phoxinus phoxinus* (Kestemont and Stalmans, 1992). Similarly, a high rate of malformation was found in carp larvae, *Ciprinus carpio*, fed a commercial dry diet and this was corrected through the *Artemia* nauplii.

Jana and Chakrabarti (1993) suggested that growth, reproductive potentials, and survival of each species are affected by the nutrient conditions of the culture media.

Hecht and Appelbaum (1987) conducted an experiment with the larvae and juveniles of *Clarias gariepinus* and concluded that growth and survival was density dependant and that live food was preferred to formulated feed.

Johnson (1965) developed an experiment with three experimental unit T1, T2 and T3. From this it was observed that the growth in terms of weight, weight gain, RG and SGR of individuals of *H. fossilis* was significantly higher in T1 where the stocking density was low compared to the treatments of T2 and T3 although same food was supplied in all the treatments at an equal ratio. The low growth rate of *H. fossilis* in treatment T2 and T3 appeared to be related with higher densities and increased competition for food and space and an inverse relationship with in the stocking density provided that space-limiting effects operate on the population.

Haque and Barua (1989) found that non-live feeds (fish meal and wheat flour) were not at all suitable for the larvae of *Heteropneustes fossilis*, while live food (tubificid worms) resulted in the best growth and survival.

Ghyeas (1998) studied the effects of three feeds viz., tubificid worms, a formulated feed and a commercial nursery feed on growth and survival of *Heteropneustes fossilis* larvae. In this case also, growth and survival of larvae fed tubificid worms gave the best result.

According to Hirano and Hanyu (1990) all developmental stages of *Clarias gariepinus* can adapt to artificial feeds, however, the best growth was obtained by larvae fed on live feed.

Mahmood, *et al.* (2004) conducted a study to assess the suitability of different larval feeds for larvae/fry of climbing perch, *Anabas testudineus*. The experiment was designed with 4 treatments, each with 3 replications and feeds was assigned to

different treatments viz. T1 (*Artemia nauplii*), T2 (tubificid worms), T3 (rotifer powder) and T4 (zooplankton). The larvae fed tubificid worms had significantly highest ($P < 0.05$) growth (percent length gain 237.80 ± 2.09 , percent weight gain 2040.10 ± 17.82 , specific growth rate 13.61 ± 0.01) and survival ($61.0 \pm 2.0\%$), followed by *Artemia nauplii*, zooplankton and rotifer powder. Therefore, tubificid worms may be suggested for feeding climbing perch larvae upto stockable size.

Comparatively better performance in growth and survival of fish larvae fed live feed was also observed by Wilson, (1991) in turbot (*Scophthalmus maximus*), milkfish (*Chanos chanos*), striped bass (*Morone spp.*) and barramundi (*Lates calcarifer*).

Kanazawa (1991) also commented that live feed is preferable for larval nutrition than non-live feeds.

Dabrowski (1984) and Dabrowski *et al.* (1987) reviewed the feeding of fish larvae, and pointed out that fish larvae are susceptible to dietary deficiency in more spectacular ways than juveniles and adults, he noted that amino acids in live foods are catabolized at a lower rate and therefore used to a greater extent for protein synthesis than amino acids from artificial diets.

Kamruzzaman *et al.* (2013) reported that prepared fish feed are found to be effective for the better growth and culture of the experimental shingi fish (*H. fossilis*). The fish feed containing 35% protein is the best feed for better growth.

Although the objective of the present study is to determine the particular level of salinity stress tolerance, efforts have been made of consult a few available literatures on the effect of salinity stress tolerance of some marine and fresh water fishes.

There have been considerable studies about the effect of salinity on growth and survival for different fishes. Although present study was conducted to determine particular salinity stress tolerance for Shing, efforts have been also made of consult a few available literatures on salinity stress tolerance of some marine and fresh water species.

Morgan and Iwama (1991) reported that the Environmental factors such as salinity strongly influence fish growth.



Likongwe *et al.* (1996) found that feed conversion efficiencies and protein efficiency ratios were highest at 32°C and 8000 ppm salinity and lowest at 28 °C and 16000 ppm salinity.

Kelly *et al.* (1999) found that salinity did not affect growth of black sea bream after 42 days of culture but did have an effect on growth after 84 days of culture. Furthermore, most researchers compared effects of salinities below, close to and above isosmotic levels on fish osmoregulation and/or growth.

Watanabe *et al.* (2007) observed that growth and survival in fish are not affected at different salinity levels when temperature exceeds 27°C but salinity has pronounced effect at temperatures below 25°C.

Iqbal *et al.* (2010) conducted a study to investigate the effect of different salinity levels on growth performance and survival of Nile tilapia (*Oreochromis niloticus*). Four salinity levels i.e. 1600 ppm in Treatment I (TI), 2400 ppm in TII, 3200 ppm in TIII and 4000 ppm in TIV were maintained while in control (T0) the salinity level was 800 ppm. Results indicate that the higher salinity (4000 ppm) levels have pronounced effect on fish growth which might be due to improved osmoregulation.

Pullin and McConnell (1982) reported that Tilapia is one of the important fish species which has several good qualities and can face wide range of salinity and other environmental conditions and can grow well in water salinities ranging from 11 ppm to 29000 ppm, tolerate temperatures between 8 to 42° C and can survive in low dissolved oxygen (DO) levels (0.1 ppm).

Kasim (1983) conducted an experiment to determine of salinity tolerance of certain freshwater fishes. Five species—fingerlings of freshwater mullet *Rhinomugil corsula* (Hamilton) (6.77 ± 1.11 cm; 2395.25 ± 1225.73 mg), fry of mrigal *Cirrhinus mrigala* (Hamilton) (1.26 ± 0.09 cm; 15.97 ± 4.09 mg), fringed carp *Labeo fimbriatus* (Bloch) (1.76 ± 0.19 cm; 41.76 ± 15.98 mg), common carp *Cyprinus carpio* Linnaeus (2.24 ± 0.22 cm; 109.43 ± 36.15 mg) and hatchlings of *Sarotherodon mossambica* Peters (0.77 ± 0.07 cm; 7.58 ± 1.06 mg)—were used for this experiment.

The result revealed that among the five species, the fingerling of *R. corsula* tolerates salinity better than the remaining four species at the same temperature (30°C).

To survive total seawater, the hatchlings could not survive beyond 7.07 ‰ (S) owing to its lack of well-developed osmoregulatory mechanism. Among carps, common carp tolerates salinity better than mrigal and *L. fimbriatus*. Mrigal is the least tolerant among all the species.

Result also reflected that in the case of euryhaline *R. corsula* and *S. mossambica* the salinity acclimation did not bring about marked change in the tolerance limit among the stenohaline carp. *L. fimbriatus*. The stenohaline carps have comparatively low tolerance and adaptability to salinity.

Schofield *et al.* (2011) conducted an experiment to determine survival, growth and reproduction of non-indigenous Nile tilapia. The stated that Salinity and temperature are environmental variables that limit the spread of many non-native fishes. They hypothesised that combinations of temperature and salinity will interact to affect survival, growth, and reproduction of Nile tilapia, *Oreochromis niloticus*, introduced into Mississippi, USA.

They conducted acute and chronic salinity tolerance experiments. For the acute experiment, tilapia were transferred from freshwater directly into salinities of 5, 10, 15, 20, 25, 30 and 35 ppt and held for a period of seven days at 25°C. Fish were held in individual containers and checked hourly for the first 8 hours, then daily for the next 6 days.

The tolerance of Nile tilapia to a chronic change in salinity was evaluated by exposing individuals to progressively increasing salinity (5 ppt per week) until all treatments reached their target salinity (0 [control], 10, 20, 30, 40, 50, 60, 70 and 80 ppt). Upon reaching their target salinities, the treatments were held for a minimum of 30 days.

Nile tilapia acutely transferred from freshwater to salinities up to 20 ppt showed good survival for at least 7 days. Nile tilapia acutely transferred from freshwater to salinities up to 20 ppt showed good survival for at least 7 days. Fish gradually

transferred at a rate of 5 ppt per week survived at salinities up to 40 ppt for at least 55 days (Schofield *et al.*, 2011).

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Chapter 3

Materials and Methods

3. Materials and Methods

The experiment was started on 18 June and continued up to 20 September 2013 in the aquarium ($54 \times 36 \times 36 \text{ cm}^3$ in size) in the wet fish laboratory under Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna.

3.1. Fry collection and rearing

Thirty days old fry of Shing (*Heteropneustes fossilis*) was collected from commercial hatchery of Chachra in Jessore. They were transferred in a large aquarium of wet fish laboratory and conditioned for two days before starting the experiment.

3.2. Water quality management

Prior to stocking of fry, each of the aquarium was cleaned up and prepared with all the facilities necessary to run the experiment efficiently. Each aquarium was fitted with a submerged filter and an air diffuser. Submerged filter kept the water clean. About three fourth ($3/4$) of the water was changed once per week. Water exchange was done by siphoning and removed feces, uneaten feed and the dead juvenile every day. Adhered dirt inside the aquarium walls and shelter pipe was cleaned also once a week. Continuous aeration was ensured for rearing throughout the experimental periods. During the experimental period, temperature, dissolved oxygen (DO), pH and Ammonia (NH_4) of water in larval rearing system was monitored regularly. Temperature reading was taken with a Celsius thermometer, dissolved oxygen (DO) of water was measured by a digital DO meter and pH reading was taken with the help of a portable pH meter.

3.3. Experimental procedure for comparing suitability of larval feed

3.3.1. Stocking

Thirty days old Shing (*Heteropneustes fossilis*) of initial total length of $2.95 \pm 0.12 \text{ cm}$ and weight $0.74 \pm 0.01 \text{ g}$ were stocked in aquarium ($54 \times 36 \times 36 \text{ cm}^3$) containing 30 liter of water at a stocking density of 30 fry per aquarium. During the study period, the shing fry were fed with four different types of feed such as *Tubifex* spp. (T_1), commercial feed (T_2), snail meat (T_3) as well as commercial feed and snail meat on alternative day (T_4).

There were three replications of each treatment and larvae were reared for 30 days.

3.3.2. Feed collection

Commercial feed and live *Tubifex* were purchased from the local aquarium shops at Moilaputa in Khulna. Fresh *Tubifex* were kept under the dropped water coming from the basin tap in the wet laboratory of Khulna university, Khulna. Thus, the *Tubifex* remained live and fresh until five or six days. Before feeding, live snails were collected from the shrimp farmer. Snail meat were chopped into small pieces by blade and scissor and preserved in refrigerator in a form of small cube. Before feeding, each snail meat cube was thawed and given to the fish.

3.3.3. Feeding frequency

The feeding frequency was 2 times per day (8.30am and 8.30 pm) at their satiation level. Before introducing feed for the next feeding, previous uneaten feeds and feces in aquarium were removed by siphoning using a plastic pipe.

3.3.4. Protein content of feed and juvenile

The level of protein (%) in samples was determined according to AOAC (1980). In brief, the sample was digested with concentrated Sulphuric acid (H_2SO_4) and solvent followed by distillation with 40% NaOH, zinc powder and distilled H_2O . At the end of the experiment, protein content of reared fishes was also determined.

3.4. Experiental Procedure for determining salinity stress tolerance

3.4.1. Determining of salinity level for direct stocking

In this stage of experiment *H. fossilis* fry were cultured in aquarium with six different salinities 0, 5, 8, 10, 12 and 15 ppt for 30 days to determine the optimum salinity level for direct stocking. At this time, larvae were not acclimatized in salinity gradually. Fry were directly exposed to the salinity 0, 5, 8, 10, 12 and 15 ppt directly from 0ppt. Brine with 90 ppt, collected from the local hatchery were used for acquiring the target salinity level. All other parameters were maintained such a way that all parameter were favorable to the fish and fish don't feel any stress for other parameters.

In this stage, Thirty days old Shing (*Heteropneustes fossilis*) of initial total length of $4.4 \pm 0.3\text{cm}$ and weight $1.59 \pm 0.11\text{g}$ were stocked in aquarium ($54 \times 36 \times 36\text{ cm}^3$) containing 30 liter of water at a stocking density of 30 fry per aquarium. The larvae fed with the best feed from the previous experiment.

3.4.2. Survivability due to gradual increasing salinity level

Finally, salinity was increased gradually starting from 0 to 15ppt in every 3 days. Same larvae were reared in different salinity level. Survivability was measured for each salinity level.

3.5. Growth performance

The growth of larvae was measured every seven days interval for both experiments. The following parameters were used to evaluate larval growth.

3.5.1. Measurement of length and weight

Sampling was done by the scoop net and ten fishes were caught randomly from each aquarium and then length and weight of the individual fish were measured. Weight and length were taken by an electric balance and length by a measuring scale.

3.5.2. Weight gain

Weight gain of the larvae was calculated by the following formula (Sandifer and Smith, 1974)

Weight gain= Mean final weight(g) - Mean initial weight (g)

3.5.3. Specific growth rate

According to Elangovan and Shim (2000), the specific growth rate (%) of larvae was calculated

$$\text{SGR(\%)} = \{(\text{Ln final weight (g)} - \text{Ln initial weight (g)})/\text{Time(day)}\} \times 100$$



3.5.4. Survival rate

Each dead fish were recorded and removed everyday (if necessary) prior to feeding. The survival rate (%) was calculated by using the following formula (Mahmood *et al.*, 2004).

$$\text{Survival rate} = \frac{\text{Number of total live fish}}{\text{Total number of stocked fish}} \times 100$$

3.6. Statistical analysis

Data are reported as arithmetic mean $\pm$ standard deviation (SD). After appropriate transformation (arcsine or logarithm), one way analysis of variance (ANOVA) was applied on the data to assess the treatment effect. All the statistical analyses were done on a computer using statistical software package SPSS (version 7.5).

Chapter 4

Results

4. Results

For the determination of larval food, four types of feed combinations were used. Among them, highest growth was found from the treatments who fed with *Tubifex* spp (T1). Interestingly, the fish fed with snail meat and commercial feed on alternative day (T4) also gained competitive weight and length. But the lowest growth was recorded from the fish who fed with commercial feed (T2) only. In this experiment, particular salinity stress tolerance also determined for the *Heteropneustes fossilis*. For the determination of direct stocking salinity level, the larvae were exposed to different salinity level such as 5ppt, 8ppt, 10ppt, 12ppt and 15ppt directly from 0ppt. The result reveals that, 10ppt, 12ppt and 15ppt are lethal salinity level for Shing larvae. About 70% survivability were recorded in 8ppt salinity that is quite satisfactory.

The longest larvae was measured in T1 (8.7 ± 0.44 cm) and the lowest in T2 (6.6 ± 0.17 cm). The second longest length was achieved in T4 (7.1 ± 0.40 cm) (Appendix-A 5). Total length were significantly varied ($P < 0.05$) in each treatment (Appendix-A 6).

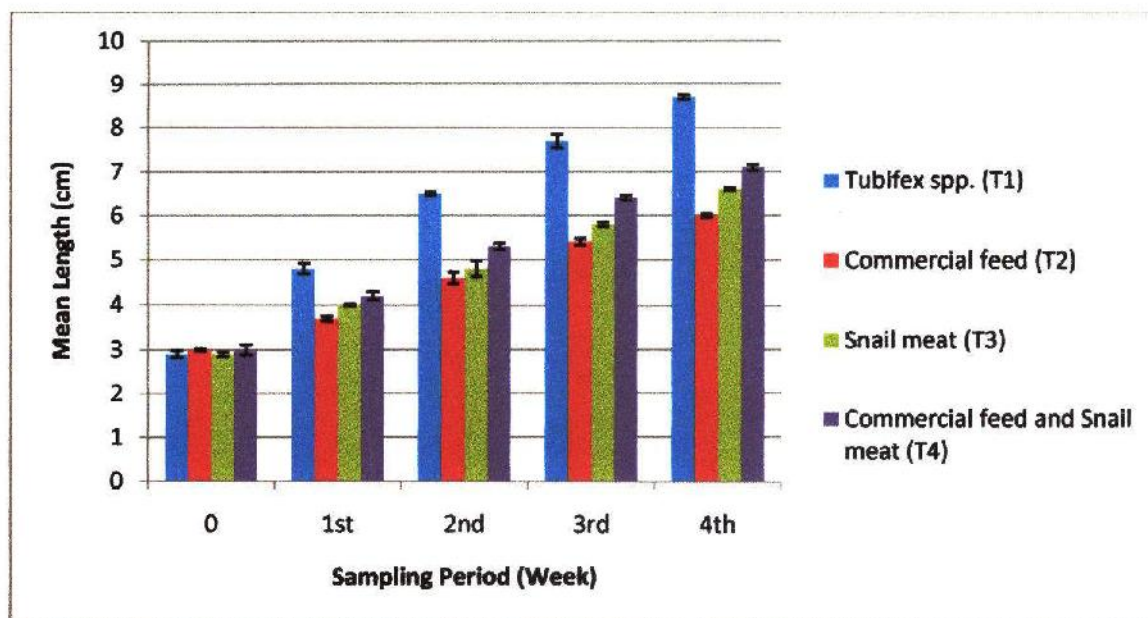


Fig. 1: Cumulative growth of *H. fossilis* larvae in terms of length during

The highest weight of larvae was measured in T1 ($3.35 \pm 0.26\text{gm}$) and the lowest in T2 ($1.86 \pm 0.07\text{gm}$). The second highest weight was achieved in T4 ($2.58 \pm 0.16\text{gm}$) (Appendix-A 7). Cumulative weight were significantly varied ($P < 0.05$) in each treatment. (Appendix-A 8).

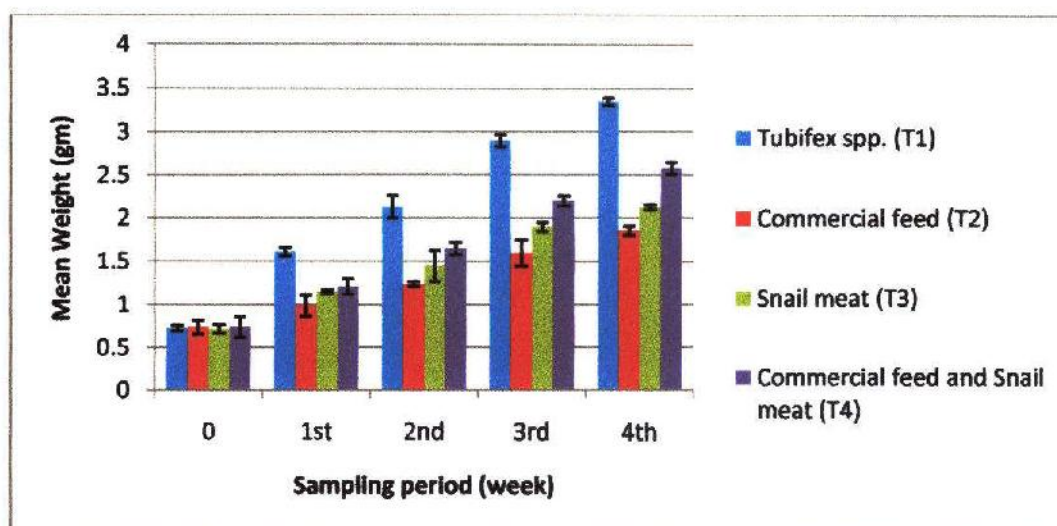


Fig. 2: Cumulative growth of *H. fossilis* larvae in terms of weight during

The value of average weight gain in T1, T2, T3 and T4 were 1.45g, 0.75g, 0.925g and 1.025g respectively. Highest weight gain average was found in T1 and lowest weight gain average was found in T4. (Appendix-A 8).

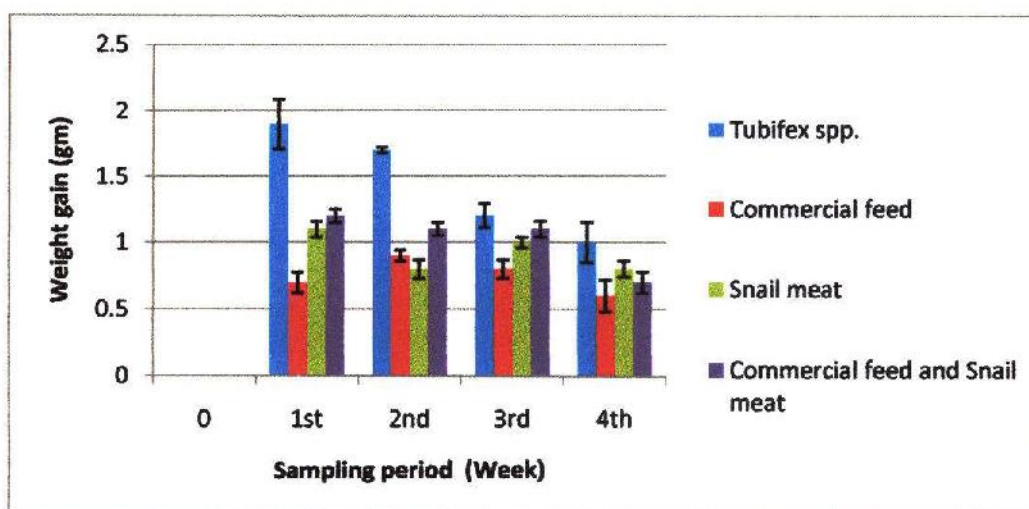


Fig. 3: Weight gain of *H. fossilis* larvae during the study period

The feeding treatments showed pronounced effects on the specific growth rate of *Heteropneustes fossilis* larvae. The specific growth rate of larvae in T1, T2, T3 and T4 were 3.66 ± 0.16 , 2.31 ± 0.12 , 2.74 ± 0.13 , 2.87 ± 0.09 . Highest specific growth rate was found in T1, whereas lowest specific growth rate was found in T2. The result reveals that the second highest specific growth rate was found in T4.

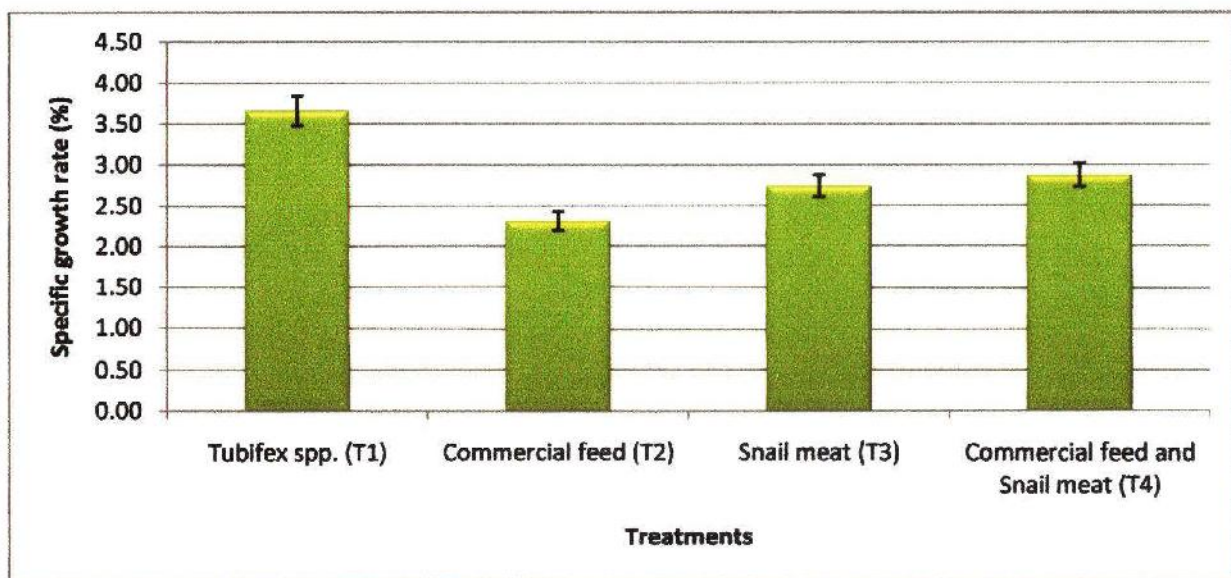


Fig. 4: Specific growth rate (%) of *H. fossilis* larvae among the treatments.

Water quality parameter is an important consideration. That's why temperature, dissolve oxygen (DO), pH and ammonia ion were monitored in every seven days interval during study period. The highest DO was recorded in T4 (7.92 ± 0.56) and lowest in T2 (7.5 ± 0.48). Ammonia, pH and temperature level was recorded almost equal in each treatment. Statistical analysis showed that there is no significant difference ($P > 0.05$) in DO, pH, ammonia ion and temperature among different treatment. (Appendix A 1, 2, 3, 4).

Table 1: The following table shows the water quality parameters among the treatments during the experimental period. Three replications were considered for each treatment. Each value represents Mean $\pm$ SD.

Parameters	Treatments			
	Tubifex spp.(T1)	Commercial feed (T2)	Snail meat (T3)	Commercial feed and Snail meat (T4)
DO (mg/l)	7.7 $\pm$ 0.51	7.5 $\pm$ 0.48	7.8 $\pm$ 0.50	7.92 $\pm$ 0.56
pH	8.1 $\pm$ 0.15	8.0 $\pm$ 0.10	8.0 $\pm$ 0.10	8.1 $\pm$ 0.13
Amonia (mg/L)	0.097 $\pm$ 0.062	0.073 $\pm$ 0.071	0.048 $\pm$ 0.069	0.056 $\pm$ 0.056
Temparature	28.76 $\pm$ 0.51	28.66 $\pm$ 0.46	28.8 $\pm$ 0.51	28.86 $\pm$ 0.46

Proximate composition analysis of fish larvae was carried out to determine protein content in terms of wet weight basis. Before rearing, the average protein (%) of fish selected for T1, T2, T3 and T4 were 13.12 $\pm$ 0.88, 13.44 $\pm$ 0.67, 12.61 $\pm$ 1.15 and 12.91 $\pm$ 1.22 respectively (Appendix-A 10). After 30 days rearing, the average protein (%) for T1, T2, T3 and T4 were found 19.23 $\pm$ 0.69, 14.75 $\pm$ 0.32, 16.37 $\pm$ 0.27 and 17.27 $\pm$ 1.06 respectively (Appendix-A 11). Significant differences ($P < 0.05$) of protein were observed among T1, T2, T3 and T4. (Appendix- A 12).

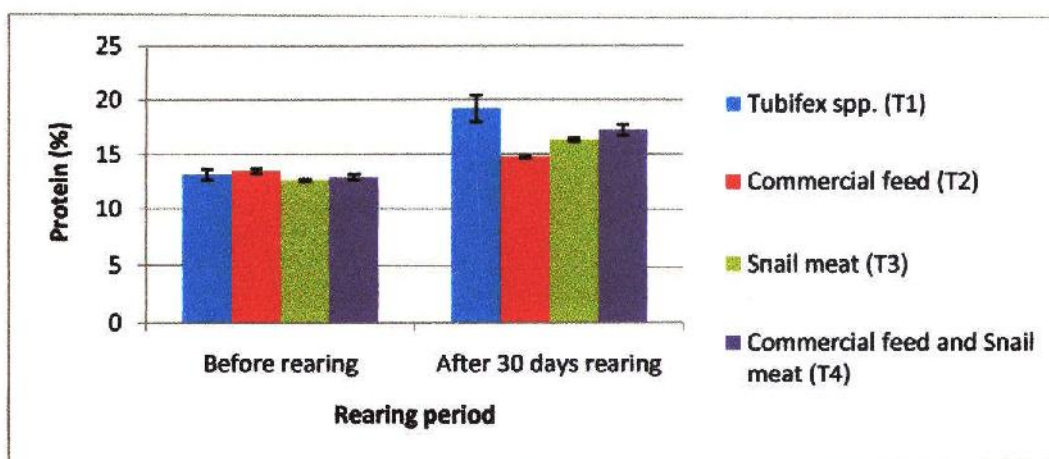


Fig. 5: Protein (%) of *H. fossilis* larvae before and after rearing

To determine acute salinity level of the *Heteropneustes fossilis* larvae, they were exposed to particular salinity from 0ppt directly. The larvae showed good performance at 0ppt, 5ppt salinity. At 8ppt salinity level, the survivability was found 69% that was satisfactory and there was no significant difference of survivability among 0ppt, 5ppt and 8ppt. But if the fish stock at 10ppt, all fish will die within next 72 hours. The result also enumerated that 12ppt and 15ppt salinity level are completely lethal for *Heteropneustes fossilis* larvae.

Table 2: The following table shows the survivability of *Heteropneustes fossilis* larvae at different salinity level.

Types of salinity	Survivability (%)				
	1st week	2nd week	3rd week	4th week	Finally
0 ppt	100%	100%	100%	100%	100%
5ppt	100%	100%	100%	100%	100%
8ppt	92%	87%	81%	69%	69%
10ppt	All die within 72 hours	0%	0%	0%	0%
12ppt	All die within 20 hours	0%	0%	0%	0%
15ppt	All die within 9 hours	0%	0%	0%	0%

Temperature, dissolved oxygen (DO), pH and ammonia ion were monitored in every seven days interval during study period. There was no significant difference among the water quality parameter and did not affect the growth at different salinity level.

Table 3: The following table shows the water quality parameters at different salinity. Three replications were considered for each treatment. Each value represents Mean $\pm$ SD.

Parameters	Treatments		
	0ppt (T1)	5ppt (T2)	8ppt (T3)
DO (mg/l)	7.6 $\pm$ 0.36	7.4 $\pm$ 0.15	7.5 $\pm$ 0.25
pH	8.2 $\pm$ 0.16	8.1 $\pm$ 0.14	8.1 $\pm$ 0.03
Amonia (mg/L)	0.045 $\pm$ 0.034	0.067 $\pm$ 0.012	0.012 $\pm$ 0.054
Temparature	29.3 $\pm$ 0.23	29.3 $\pm$ 0.12	29.4 $\pm$ 0.56

The larvae achieved weight 3.16 ± 0.16 gm, 3.08 ± 0.15 gm and 2.13 ± 0.07 gm at 0ppt, 5ppt and 8ppt respectively. There was no significant difference in growth among 0ppt and 5ppt. But the larvae stocked at 8ppt were affected adversely with salinity and significant difference ($P < 0.05$) was found by the statistical analysis in terms of weight among different treatments (Appendix-B1).

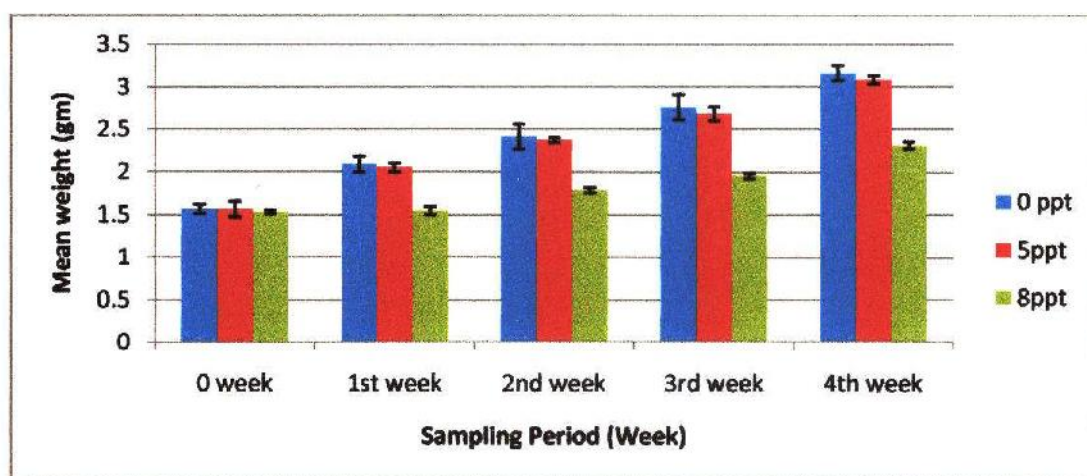


Fig. 6: Cumulative weight at different salinity

The fish achieved length, 7.7 ± 0.12 cm, 7.4 ± 0.20 cm and 6.6 ± 0.20 cm at 0ppt, 5ppt and 8ppt respectively. There was no significant difference in growth in terms of length among 0ppt and 5ppt. But the larvae stocked at 8ppt were affected adversely with salinity and significant difference ($P < 0.05$) was found by the statistical analysis in terms of length among different treatments (Appendix-B2).

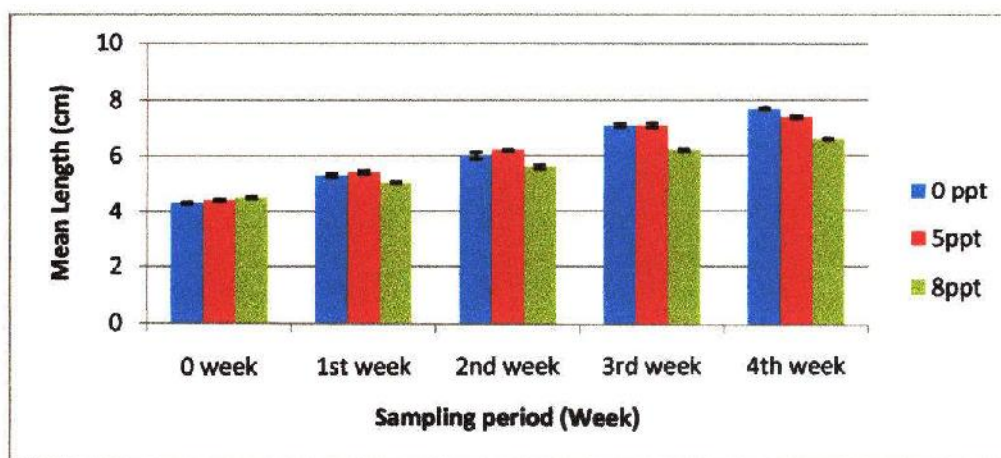


Fig. 5: Cumulative length at different salinity

Finally to determine particular salinity stress the salinity was increased gradually 1ppt in every 3 days. The result revealed 100% survivability up to 7ppt. The survivability rate was also satisfactory at 8ppt, 9ppt and 10ppt salinity level. The salinity level 13ppt and 14ppt were recognized as lethal salinity level.

Table 4: Survivability of *Heteropneustes fossilis* larvae at different salinity level.

[illegible]

Finally to determine particular salinity stress the salinity was increased gradually 1ppt in every 3 days. The result revealed 100% survivability up to 7ppt. The survivability rate was also satisfactory at 8ppt, 9ppt and 10ppt salinity level. The salinity level 13ppt and 14ppt were recognized as lethal salinity level.

Table 4: Survivability of *Heteropneustes fossilis* larvae at different salinity level.

Types of salinity	Survivability in sampling day											
	3th	6th	9th	12th	13th	14th	15th	16th	17th	18th	19th	20th
0 ppt	100%											
5ppt		100%										
6ppt			100%									
7ppt				100%								
8ppt					98%							
9ppt						97%						
10ppt							76%					
11ppt								72%				
12ppt									66%			
13ppt										42%		
14ppt											23%	
15ppt												0%

Chapter 5

Discussions



05. Discussions

Determination of appropriate feed is crucial technique in the culture of aquatic animals. To ensure aquaculture commercially viable, knowledge of induced breeding, larval rearing, grow-out and brood stock maintenance is needed. Among various stage of life, larval stage is considered one of the most sensitive phases during life cycle. The present study determined the appropriate larval feed for *Heteropneustes fossilis* with particular reference to salinity stress tolerance.

5.1 Water quality parameters

Water quality parameters for the rearing of *Heteropneustes fossilis* larvae in the experiment were not changed sharply. Each aquarium was fitted with a submerged filter and an air diffuser. Submerged filter kept the water clean and air diffuser provided dissolve oxygen in water continuously. About three fourth (3/4) of the water was changed once per week. Water exchange was done by siphoning and removed feces, uneaten feed and the dead larvae every day. Water temperature, pH, dissolve oxygen and ammonia were in the acceptable range which is supported by the other authors (Boyd, 1982; Jhingram and Pullin, 1985; Rahman *et al.*, 1982).

Temperature is one of the most important physico-chemical parameters, which directly influences the physical, chemical and biological nature of water body. The water temperature of this study was range between 28°C to 29.7°C.

The pH in water body generally regulates considerably the water chemistry. Any sudden fluctuations causes death of many aquatic species. On the other hand, low pH reduces ammonia toxicity (Whitfield, 1974) but mobilize metals, including iron and aluminium, which can reduce yields in aquaculture ponds (Simpson *et al.*, 1983). Low pH also can reduce natural productivity by reducing the availability of nutrients (Alasbaster and Lloyd, 1980). The range of water pH in this present study varied between 7.9 to 8.2.

Dissolve oxygen in the experimental aquarium was in productive level. Banergea (1967) reported 7.0 mg/l of dissolve oxygen in water body is good for productivity whereas the range from 3.0-5.0 mg/l is unproductive for fish culture. George (1961)

stated that the productive range of dissolved oxygen is 4.5 -9.9 mg/l for fish culture. Ali *et al.* (1982) stated that the favorable DO range is 7.2 – 12.5 mg/l for fish culture. In the present study, DO level varied among the treatments from 6.9 to 8.9 mg/l. From the overall findings of water quality parameter it can be said that temperature, pH, and DO in all treatments were in optimum level for the *Heteropneustes fossilis* larvae.

5.2 Growth Performance

The growth and survival rates of larvae were highly influenced by different larval feeds. As evident from Fig1 and Fig 2, the present study determines that the larvae fed with *Tubifex* showed highest growth performance (Length- 8.7 ± 0.44 cm and weight- 3.35 ± 0.26 gm). At the same time, the second highest growth performance was recorded in T4 (snail meat and commercial feed were fed at alternative day). The measured length and weight were 7.1 ± 0.40 cm and 2.58 ± 0.16 gm respectively. The highest weight gain, specific growth rate and survivability also found for larvae fed *Tubifex* (Fig.3 and Fig.4).

Hecht and Appelbaum (1987) conducted an experiment with the larvae and juveniles of *Clarias gariepinus* and also concluded that live food was preferred to formulated feed and suitable for fish growth. Haque and Barua (1989) found that non-live feeds (fish meal and wheat flour) were not at all suitable for the larvae of *Heteropneustes fossilis*, while live food (tubificid worms) resulted in the best growth and survival.

Fermin and Boliver (1991) reported that the specific growth rate of *Clarias macrocephalus* larvae fed live feed was higher than those fed non-live food. Bairage *et al.* (1988) tested the effect of *Artemia nauplii*, zooplankton and a formulated artificial feed on *Clarias batrachus* larvae for a period of four weeks and found live feed (*Artemia nauplii*) as the best feed with regard to growth and survival. According to Rahman *et al.* (1974), the growth and survival of the larvae of *C. batrachus* was better in larvae fed tubificid worms than those fed non-live feeds, egg yolk in particular. Haque and Barua (1989) found that non-live feeds (fish meal and wheat flour) were not at all suitable for the larvae of *Heteropneustes fossilis*, while live food (tubificid worms) resulted in the best growth and survival.

In the present study the combine feeding showed better result than the commercial feeding or snail meat feeding only. *Heteropneustes fossilis* larvae are mainly carnivorous so that they prefer live feed. In that sense, the larvae fed with snail meat should achieve second highest growth. But the present study reflects different result that is very interesting output in this experiment.

The highest protein content was found in *Tubifex* consumed larvae and the lowest was found in commercial feed consumed larvae. After 30 days rearing, the average protein (%) for T1, T2, T3 and T4 were found 19.23 ± 0.69 , 14.75 ± 0.32 , 16.37 ± 0.27 and 17.27 ± 1.06 respectively (Appendix-A 11). Significant differences ($P < 0.05$) of protein were observed among T1, T2, T3 and T4. (Appendix- A 12). The specific growth rate of larvae in T1, T2, T3 and T4 were 3.66 ± 0.16 , 2.31 ± 0.12 , 2.74 ± 0.13 , 2.87 ± 0.09 . Highest specific growth rate was found in T1, whereas lowest specific growth rate was found in T2 (Fig. 4).

5.3. Salinity Stress Tolerance (Direct exposing)

Effects of salinity on fish growth vary greatly among fish and among salinity ranges tested (Boeuf and Payan, 2001). *Heteropneustes fossilis* larvae were exposed to particular salinity from 0ppt directly to determine optimum stocking salinity. Good performance was found at 0ppt, 5ppt salinity. At 8ppt salinity level, the survivability was found 69% that was satisfactory and there was no significant difference of growth among 0ppt, 5ppt and 8ppt. Kasim (1983) conducted an experiment on Salinity tolerance of certain freshwater fishes and concluded that mrigal is the least tolerant (3.5454 (S)), the common carp the most tolerant (7.07 %o). *L. fimbriatus* being intermediate (7.07 %o). The larvae achieved weight 3.16 ± 16 gm, 3.08 ± 15 gm and 2.13 ± 07 gm at 0ppt, 5ppt and 8ppt respectively. There was no significant difference in growth among 0ppt and 5ppt. But the larvae stocked at 8ppt were affected negatively with salinity and significant difference ($P < 0.05$) was found by the statistical analysis in terms of weight among different treatments (Appendix-B1). The larvae achieved length of 7.7 ± 0.12 cm, 7.4 ± 0.20 cm and 6.6 ± 0.20 cm at 0ppt, 5ppt and 8ppt respectively. There was no significant difference in growth in terms of length among 0ppt and 5ppt. But the larvae stocked at 8ppt were affected negatively with salinity

and significant difference ($P < 0.05$) was found by the statistical analysis in terms of length among different treatments (Appendix-B2).

5.4. Salinity Stress Tolerance (Gradual exposing)

The salinity was also increased gradually to determine maximum salinity stress tolerance. In case of gradual increasing of salinity, the survivability at 0 to 7ppt, 8ppt, 9ppt, 10ppt, 11ppt and 12ppt were found 100%, 98%, 97%, 76% , 72% and 66% respectively. The survivability was quite satisfactory up to 12ppt. But at more than 12ppt salinity, it affects the fish growth and survivability negatively. So, the salinity level above 12ppt considered as lethal salinity and is not appropriate for *H. fossilis* culture. However, gradual exposing helps larvae to adapt the salinity. That's why, *Heteropneustes fossilis* larvae tolerated more salinity stress in case of gradual increasing rather than direct exposing. Schofield *et al.* (2011) found similar result in his experiment on non-indigenous Nile tilapia. Nile tilapia acutely transferred from freshwater to salinities up to 20 ppt showed good survival for at least 7 days. Nile tilapia acutely transferred from freshwater to salinities up to 20 ppt showed good survival for at least 7 days. Fish gradually transferred at a rate of 5 ppt per week survived at salinities up to 40 ppt for at least 55 days.

Chapter 6

Conclusion

6. Conclusion

The present study was conducted to determine larval feed of *Heteropneustes fossilis* with particular reference to salinity stress tolerance. The result showed that variation of diet considerably influenced the growth of larvae during 30 days experimental period. *Tubifex* fed juvenile consistently showed the highest final mean length and mean weight. The second highest growth comparing with *Tubifex* was gained by the larvae who fed commercial feed and snail meat both alternatively. Although *Tubifex* is very costly and not available in local market, use of commercial feed and snail meat will be good alternatives. To determine salinity stress tolerance fish larvae were cultured into different salinity level. There is no significant difference among the fish stocked at 0ppt and 5ppt. But the fish stocked at 8ppt has significant difference with the fish stocked at 0ppt and 5ppt. That's why, the present study reflects that 0 to 5ppt is best, 6 to 8 ppt is better and above 10ppt is lethal for direct stocking of fish larvae. The salinity was also increased gradually to determine maximum salinity stress tolerance. In case of gradual increasing of salinity, the survivability at 0 to 7ppt, 8ppt, 9ppt, 10ppt, 11ppt and 12ppt were found 100%, 98%, 97%, 76% , 72% and 66% respectively. Until this salinity level reaches to 12ppt, the survivability was quite satisfactory. This salinity level (12ppt) can be marked as critical salinity level. The salinity level above 13ppt can be considered as lethal for *H. fossilis* culture.

Heteropneustes fossilis is an important native species which has both attractive market value and high consumer preference. The present study determined the appropriate larval feed for getting good production at a shorter culture period. At the same time, the present study explored that this fish can tolerate 12ppt salinity. In some area of southern part of Bangladesh, the salinity level varies 0 to 15ppt. About five or six months in a year salinity level remains 0 to 6ppt. That is a good opportunity for Shing culture. However, the present study was conducted in aquarium under laboratory condition. So, more field based study is needed to fine tune the result of salinity stress tolerance. After that this knowledge can be transferred to the farmer.

Chapter 7

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7. References

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Chapter 8

Appendices

8. Appendices – A

Appendix-A 1: Statistical analysis of the total pH among the treatments

ANOVA

pH					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.015	3	.005	1.406	.310
Within Groups	.028	8	.003		
Total	.043	11			

Homogeneous

pH

		N	Subset for alpha = 0.05
Treatment			1
Tukey HSD ^a	Commercial feed	3	7.993
	Snail meat	3	8.027
	Tubifex	3	8.060
	Snail meat and commercial feed	3	8.087
	Sig.		.288
Duncan ^a	Commercial feed	3	7.993
	Snail meat	3	8.027
	Tubifex	3	8.060
	Snail meat and commercial feed	3	8.087
	Sig.		.107
Scheffe ^a	Commercial feed	3	7.993
	Snail meat	3	8.027
	Tubifex	3	8.060
	Snail meat and commercial feed	3	8.087
	Sig.		.356

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix-A 2: Statistical analysis of the total Dissolve oxygen (DO) among the treatments

ANOVA

DO

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.265	3	.088	2.101	.178
Within Groups	.337	8	.042		
Total	.602	11			

DO

		N	Subset for alpha = 0.05
Treatment			1
Tukey HSD ^a	Commercial feed	3	7.513
	Tubifex	3	7.707
	Snail meat	3	7.800
	Snail meat and commercial feed	3	7.920
	Sig.		.148
Duncan ^a	Commercial feed	3	7.513
	Tubifex	3	7.707
	Snail meat	3	7.800
	Snail meat and commercial feed	3	7.920
	Sig.		.052
Scheffe ^a	Commercial feed	3	7.513
	Tubifex	3	7.707
	Snail meat	3	7.800
	Snail meat and commercial feed	3	7.920
	Sig.		.198

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.



Appendix-A 3: Statistical analysis of the total Temperature among the treatments.

ANOVA

Temperature					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.043	3	.014	1.576	.270
Within Groups	.073	8	.009		
Total	.117	11			

Homogeneous

Temperature

		N	Subset for alpha = 0.05
Treatment			1
Tukey HSD ^a	Commercial feed	3	28.7000
	Tubifex	3	28.7667
	Snail meat	3	28.8000
	Snail meat and commercial feed	3	28.8667
	Sig.		.222
Duncan ^a	Commercial feed	3	28.7000
	Tubifex	3	28.7667
	Snail meat	3	28.8000
	Snail meat and commercial feed	3	28.8667
	Sig.		.080
Scheffe ^a	Commercial feed	3	28.7000
	Tubifex	3	28.7667
	Snail meat	3	28.8000
	Snail meat and commercial feed	3	28.8667
	Sig.		.283

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix-A 4: Statistical analysis of the total Ammonia ion among the treatments.

ANOVA

Amonia

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.004	3	.001	1.811	.223
Within Groups	.006	8	.001		
Total	.011	11			

Homogeneous

Amonia

		N	Subset for alpha = 0.05
Treatment			1
Tukey HSD ^a	Snail meat	3	.04840
	Snail meat and commercial feed	3	.05557
	Tubifex	3	.07270
	Commercial feed	3	.09737
	Sig.		.220
Duncan ^a	Snail meat	3	.04840
	Snail meat and commercial feed	3	.05557
	Tubifex	3	.07270
	Commercial feed	3	.09737
	Sig.		.079
Scheffe ^a	Snail meat	3	.04840
	Snail meat and commercial feed	3	.05557
	Tubifex	3	.07270
	Commercial feed	3	.09737
	Sig.		.281

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix-A 5: Average cumulative growth of *Heteropneustes fossilis* in terms of Length (cm) gain during study period

Treatments	Cumulative growth in length (cm)				
	0 week	1 st week	2 nd week	3 rd week	4 th week
Tubifex (T ₁)	2.9±0.10	4.8±0.45	6.5±0.36	7.7±0.46	8.7±0.44
Commercial feed (T ₂)	3±0.6	3.7±0.20	4.6±0.20	5.4±0.17	6.0±0.26
Snail meat (T ₃)	2.9±0.20	4.0±0.21	4.8±0.35	5.8±0.15	6.6±0.17
Commercial feed and snail meat (T ₄)	3±0.12	4.2±0.42	5.3±0.36	6.4±0.47	7.1±0.40

Appendix-A 6: Statistical analysis of the total length (cm) among the treatments.

ANOVA

Length

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.063	3	4.021	35.478	.000
Within Groups	.907	8	.113		
Total	12.969	11			

Length

Treatment		N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD ^a	Commercial feed	3	6.0000		
	Snail meat	3	6.6000	6.6000	
	Snail meat and commercial feed	3		7.1333	
	Tubifex	3			8.7000
	Sig.		.208	.285	1.000
Duncan ^a	Commercial feed	3	6.0000		
	Snail meat	3	6.6000	6.6000	
	Snail meat and commercial feed	3		7.1333	
	Tubifex	3			8.7000
	Sig.		.061	.088	1.000

Appendix-A 7: Average cumulative growth of *Heteropneustes fossilis* in terms of weight(gm) gain during study period

Treatments	Cumulative growth in weight (gm)				
	0 week	1 st week	2 nd week	3 rd week	4 th week
Tubifex (T ₁)	0.73±0.01	1.61±0.05	2.13±0.21	2.9±0.22	3.35±0.26
Commercial feed (T ₂)	0.74±0.02	0.99±0.15	1.24±0.12	1.6±0.17	1.86±0.07
Snail meat (T ₃)	0.72±0.02	1.15±0.11	1.45±0.14	1.9±0.07	2.13±0.10
Commercial feed and snail meat (T ₄)	0.74±0.01	1.21±0.21	1.65±0.19	2.2±0.19	2.58±0.16

Appendix-A 8: Statistical analysis of the cumulative weight (gm) among the treatments.

ANOVA

Weight

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.823	3	1.274	47.503	.000
Within Groups	.215	8	.027		
Total	4.038	11			

Weight

		N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD ^a	Commercial feed	3	1.8560		
	Snail meat	3	2.1270		
	Snail meat and commercial feed	3		2.5767	
	Tubifex	3			3.3467
	Sig.		.255	1.000	1.000
Duncan ^a	Commercial feed	3	1.8560		
	Snail meat	3	2.1270		
	Snail meat and commercial feed	3		2.5767	
	Tubifex	3			3.3467
	Sig.		.077	1.000	1.000

Appendix-A 9: Average weight gain of *Heteropneustes fossilis* during study period

Treatment	0 Week	1 st week	2 nd week	3 rd week	4 th week	Average
Tubifex spp. (T1)	0	1.9	1.7	1.2	1	1.45
Commercial feed (T2)	0	0.7	0.9	0.8	0.6	0.75
Snail meat (T3)	0	1.1	0.8	1	0.8	0.925
Commercial feed and Snail meat (T4)	0	1.2	1.1	1.1	0.7	1.025

Appendix-A 10: Protein determination (wet weight basis) of *Heteropneustes fossilis* larvae before rearing

Sample No	Treatment Name	Sample wt.	Titrant (ml)	N ₂ %	Protein%	Average protein %
4	T1(No feed)	0.412	5.7	1.937	12.11	13.12±0.88
5		0.337	5.3	2.202	13.76	
6		0.357	5.5	2.157	13.48	
1	T2 (No feed)	0.387	5.6	2.026	12.66	13.44±0.67
2		0.324	5.1	2.204	13.77	
3		0.334	5.3	2.222	13.88	
7	T3 (No feed)	0.428	6.2	2.028	12.68	12.61±1.15
8		0.376	5.9	2.197	13.73	
9		0.467	6.1	1.829	11.43	
10	T4 (No feed)	0.487	6.4	1.840	11.50	12.91±1.22
11		0.334	5.2	2.180	13.62	
12		0.354	5.5	2.175	13.59	

Appendix-A 11: Protein determination (wet weight basis) of *Heteropneustes fossilis* larvae after 30 days rearing.

Sample No	Treatment Name	Sample wt.	Titrant (ml)	N ₂ %	Protein%	Average protein %
4	Tubifex (T ₁)	0.347	7.8	3.147	19.67	19.23±0.69
5		0.362	8.1	3.133	19.58	
6		0.413	8.7	2.949	18.43	
1	Commercial feed (T ₂)	0.375	6.3	2.352	14.70	14.75±0.32
2		0.342	5.9	2.415	15.10	
3		0.369	6.1	2.314	14.46	
7	Snail meat (T ₃)	0.413	7.7	2.610	16.31	16.37±0.27
8		0.423	7.8	2.582	16.13	
9		0.378	7.2	2.667	16.67	
10	Commercial feed and snail meat (T ₄)	0.399	7.9	2.772	17.32	17.27±1.06
11		0.349	7.3	2.928	18.30	
12		0.438	8.1	2.589	16.18	

Appendix-A 12: Statistical analysis of the average protein (%) among the treatments.

ANOVA

protein					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	31.310	3	10.437	23.402	.000
Within Groups	3.568	8	.446		
Total	34.878	11			

Homogeneous

protein					
Treatments		N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD ^a	commercial feed	3	14.7533		
	snail meat	3	16.3700	16.3700	
	commercial feed and tubifex	3		17.2667	
	tubifex	3			19.2267
	Sig.		.070	.409	1.000
Duncan ^a	commercial feed	3	14.7533		
	snail meat	3		16.3700	
	commercial feed and tubifex	3		17.2667	
	tubifex	3			19.2267
	Sig.		1.000	.139	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.



Appendix-B

Appendix-B 1: Statistical analysis of weight among the treatments in different salinity (0ppt, 5ppt and 8ppt).

ANOVA

weight					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.328	2	.663	39.744	.000
Within Groups	.100	6	.017		
Total	1.427	8			

Homogeneous

weight				
	treat ment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD ^a	8ppt	3	2.3103	
	5ppt	3		3.0823
	0ppt	3		3.1613
	Sig.		1.000	.745
Duncan ^a	8ppt	3	2.3103	
	5ppt	3		3.0823
	0ppt	3		3.1613
	Sig.		1.000	.482

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix-C (Photo Gallery)

Fig-1: Aquaria setup for feeding trial



Fig-2: Shing larvae in the Aquaria.



Fig-3: PVC pipe in the aquarium for protection



Fig-4: Large aquaria for acclimatization

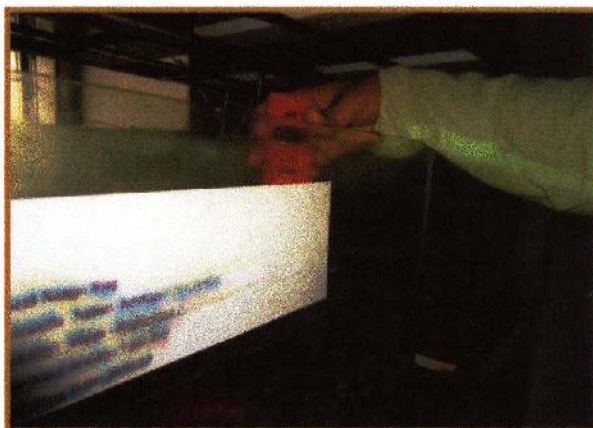


Fig-5: pH Determination with pH meter



Fig-6: Salinity determination

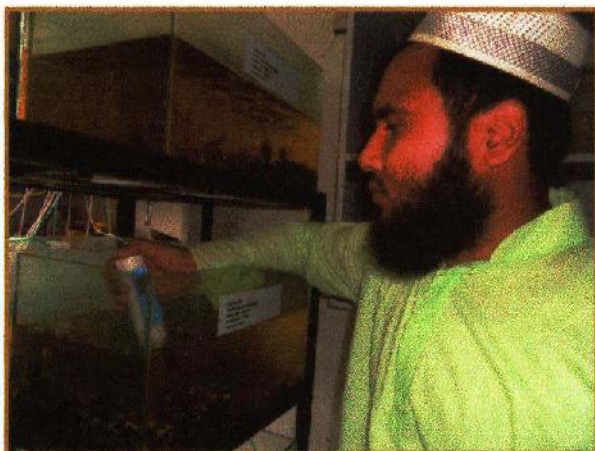


Fig-7: Dissolve Oxygen Determination



Fig-8: Overhead Tank



Fig-9: Clean Water Fill up



Fig-10: Water is changed by Siphoning

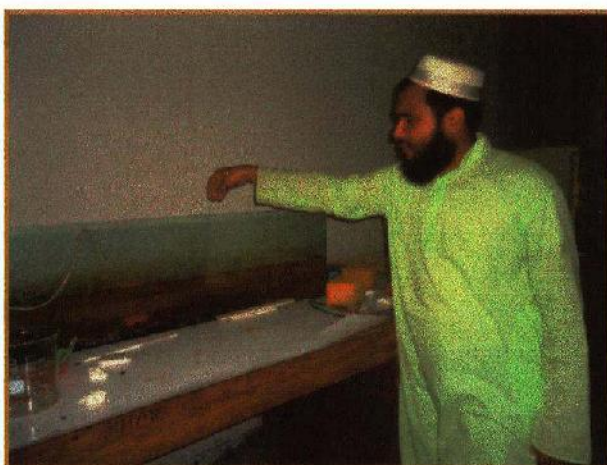


Fig-11: Feeding



Fig-12: Aquarium set up for Salinity test