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# Captive Breeding of Trichogaster lalius (Hamilton, 1822) and Trichogaster fasciata Bloch and Schneider, 1801

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# Captive Breeding of *Trichogaster lalius* (Hamilton, 1822) and *Trichogaster fasciata* Bloch and Schneider, 1801

**A Thesis**
**submitted to the University of Rajshahi**
**for the Degree of Master of Philosophy**

by

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**October, 2015**

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*Dedicated to my mother...*

# STATEMENT OF ORIGINALITY

I certify that this thesis, and the research presented within it, are the product of my own work. The guidance I received from my supervisor is acknowledged in a section dedicated to this purpose. Throughout the thesis, the ideas of other people are cited using a referencing format typical of that seen in the biological sciences. Other views and opinions given are those of the author. The thesis has not been submitted elsewhere in part or full for any degree or prize.

***(Shams Muhammad Galib)***

Researcher

***October, 2015***

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# CERTIFICATE

This is to certify that Mr. **Shams Muhammad Galib**, an M.Phil. Fellow of the Department of Fisheries, Faculty of Agriculture, University of Rajshahi, Rajshahi 6205, Bangladesh (Session: 2011-2012; Roll No. 11810), has carried out this research work on "**Captive breeding of *Trichogaster lalius* (Hamilton, 1822) and *Trichogaster fasciata* Bloch and Schneider, 1801**" under my continuous supervision and this work has not formed the basis for the award of any degree, diploma, fellowship or any other title in this university and any other university or any other institution of higher education. He fulfilled all the requirements and regulations relating to the nature and period of research. It is further certified that the entire work presented here as a thesis for the degree of **Masters of Philosophy** is based on the results of author's own investigation.

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# ABSTRACT

The study was conducted for a period of four years four years, from July 2011 to June 2015, with a view to producing offspring of two rapidly declining indigenous gourami species (*Trichogaster lalius* and *T. fasciata*) of Bangladesh in captivity. Other related issues like fecundity, larval development, breeding behavior and aquarium market potentialities were also studied. Larvae were produced under three treatments ($T_1$=without hormone; $T_2$=using hormone, PG; and $T_3$=using hormone, ovulin) in both experimental aquaria and earthen ponds. Young fishes were collected from the wild sources and reared separately at the laboratory according to their sex until breeding trial. Male and female of *T. fasciata* reached 7.94±2.36 cm (10.10±1.45 g) and 8.23±2.31 cm (11.98±1.12 g) respectively, prior to breeding trial. Whereas, male and female of *T. lalius* were 5.94±1.99 cm (7.14±1.33 g) and 6.02±2.13 cm (7.84±1.56 g) respectively. Mean individual fecundity was found 1273±516.81 (*T. fasciata*) and 160.50±26.42 (*T. lalius*). In aquaria, the highest number of offspring of *T. fasciata* (345±49.93) was obtained in the $T_3$ in 2014. Whereas, maximum number of *T. lalius* larvae (79.67±11.85) were obtained in the $T_3$ in 2013. In earthen ponds, the highest number of offspring of *T. fasciata* (1532±249.31) was obtained in the $T_1$ in 2014. Whereas, maximum number of *T. lalius* larvae (265±39.15) were obtained in the $T_1$ in 2014. ANOVA analyses have revealed that all the results found in case of $T_1$ and $T_3$ were significantly different from $T_2$ in both aquaria and earthen ponds ($p<0.05$). Half of the total newly born *T. fasciata* was observed in $T_3$ on the 10$^{th}$ day after hatching followed by $T_1$ (47% survival). In case of *T. lalius*, about one-third of the total larvae survived till the 10$^{th}$ day after hatching in $T_3$ followed by $T_1$ where about 60% larvae survived. Relationship of the breeding performance and water quality parameters were studied and described. Almost all the parameters were in suitable range for aquatic organisms in all the treatments and no statistically significant differences were recorded in maximum cases ($P>0.05$). Good demand of gouramies produced in captivity was recorded and the benefit-cost ratio was found very high. The breeding techniques in the present research could particularly be useful for both the conservation and expansion of ornamental trade of studied species.

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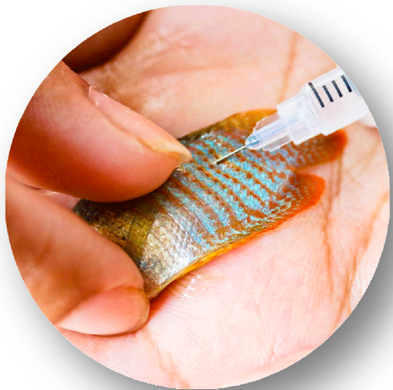
## CHAPTER ONE

# INTRODUCTION



# INTRODUCTION

## 1.1 Fisheries and Bangladesh

Being a country of rivers and floodplains with a high potential of aquatic resources, fish plays a very important role in daily life of many people in Bangladesh. In the year 2013-2014 Bangladesh has produced 3,548,115 metric tons of fishes including 2,952,730 metric tons from inland fisheries and 595,385 metric tons from marine fisheries (DoF 2015). Inland fisheries includes capture fisheries and culture fisheries (aquaculture). These two are the main contributors of total fish production from where 995,805 metric tons and 1,956,925 metric tons fish were produced (DoF 2015).

The total fish production accounts for 3.69% of GDP and the contribution to agricultural sector is 22.60% (DoF 2015). In Bangladesh, a total of 1,316,000 people are involved in fishing activities including 800,000 inland fishermen and 516,000 marine fishermen (DoF 2014). However, a recent statistics has shown that number of total fishermen (estimated) in Bangladesh is 2.0 million (DoF 2015). Also, 4.0 million people are associated with fish and shrimp/prawn farming (DoF 2015).

Fish has traditionally been a staple of the Bangladeshi diet. It plays a vital nutritional role especially in the diet to low-income rural households, accounting for 60% of animal protein intake. In Bangladesh, per capita annual fish intake is 19.30 kg (DoF 2014 and 2015) which was 17.23 kg couple of years back (DoF 2009); whereas per capita annual fish needed is 21.90 kg (DoF 2015). Though Bangladesh is provided with such potential resources, but per capita consumption rate is still lower than that of required value. Aquaculture is found as the cheapest way to supply protein in the diet of rural people.

The inland aquatic habitats of Bangladesh are rich in faunal diversity containing at least 265 species of finfish, 63 species of prawn, several species of turtles, tortoises, freshwater mussels and other living aquatic organisms (Rahman 2005).

Bangladesh is blessed with rich and extensive inland and marine fisheries resources with a wide variety of indigenous and exotic fish fauna. The soil, water and climate of Bangladesh are very favorable for inland fisheries, both open water and closed water.

Though fisheries sector is primarily for producing enough fishes for the consumption of countrymen and export, another promising venture has recently came under limelight, *i.e.* business of ornamental fish or aquarium fish. In Bangladesh, traditional use of fishes, *i.e.* as food fish, has recently being modified and some potential ornamental species are now being considered for aquarium or ornamental fish markets. The most valuable fish, based on cost per unit, weight, length are the ornamental fishes (Saxena 2003). Several research findings have revealed the present status and prospects of this ornamental fish venture in Bangladesh. Galib and Mohsin (2010 and 2011) noted that business of ornamental fish is very popular and profitable in different parts of Bangladesh and majority of the ornamental fishes that are being sold in different aquarium shops of the country are coming from Thailand. Galib *et al.* (2013a) conducted a study on aquarium fish business in Jessore district and they have recommended that indigenous potential ornamental fishes should be included to the collection of aquarium fish traders in order to get more popularity and to reduce the cost of business.

Many of these are caught in their native tropical environment and are shipped to distribute around the world. Like many other countries of the world, Bangladesh is also a place where a considerable number of ornamental fishes are found in different water bodies. Some of these potential species are listed in Table 1.1. Many species that have little or no food value to the people of Bangladesh but are very popular in aquarium trade worldwide, even in Bangladesh. Unfortunately, though these species are available in the wild of Bangladesh but are being imported from foreign countries.

**Table 1.1:** A list of potential ornamental indigenous fish species of Bangladesh

| Serial | Species | English name | Local name |
|---|---|---|---|
| 01. | *Botia dario* | Bengal loach | Rani, Bou, Putul |
| 02. | *Botia lohachata* | Y loach | Rani, Bou, Putul |
| 03. | *Lepidocephalus guntea* | Guntea loach | Gutum |
| 04. | *Parambassis lala* | Highfin glassy perchlet | Lal chanda |
| 05. | *Tetraodon cutcutia* | Ocellated pufferfish | Potka, Tepa |
| 06. | *Chelonodon patoca* | Milkspotted puffer | Potka |
| 07. | *Xenentodon cancila* | Freshwater gar | Kakila |
| 08. | *Channa punctata* | Spotted snakehead | Taki, Shati |
| 09. | *Channa orientalis* | Walking snakehead | Ghaira, Gachua |
| 10. | *Danio devario* | Dind danio | Chapchela, Debari |
| 11. | *Danio rerio* | Zebra danio | Anju |
| 12. | *Puntius phutunio* | Spotted sail barb | Phutani puti |
| 13. | *Puntius conchonius* | Rosy barb | Kanchan puti |
| 14. | *Puntius ticto* | Ticto barb | Tit puti |
| 15. | *Somileptes gongota* | Gongota loach | Pahari gutum |
| 16. | *Batia dayi* | Hora loach | Rani, Bou |
| 17. | *Hara hara* | Kosi hara | Katakunti, Titmagur |
| 18. | *Chitala chitala* | Clown knifefish | Chital |
| 19. | *Notopterus notopterus* | Bronze featherback | Foli |
| 20. | *Trichogaster chuna* | Honey gourami | Chuna khalisha |
| 21. | *Trichogaster fasciata* | Banded gourami | Khalisha |
| 22. | *Trichogaster lalia* | Dwarf gourami | Lal Khalisa |
| 23. | *Ctenops nobilis* | Frail gourami | Naftani, Madhumala |
| 24. | *Scatophagus argus* | Spotted scat | Bistara |
| 25. | *Nandus nandus* | Mud perch | Meni, Bheda, Royna |
| 26. | *Badis badis* | Badis | Napit koi, Koibandi |
| 27. | *Chanda nama* | Elongate glass perch | Nama chanda |
| 28. | *Chanda ranga* | Indian glassy fish | Ranga chanda |
| 29. | *Macrognathus aculeatus* | Lesser spiny eel | Tarabaim |
| 30. | *Mastacembelus armatus* | Zigzag eel | Baim, Sal baim |
| 31. | *Mastacembelus pancalus* | Striped spiny eel | Guchi |

Gouramies (also goumaris) are very popular in both global and domestic aquarium trades because of their brilliant colors. They are commonly known as labyrinth fish belonging to the order Perciformes (Perches) under family Osphronemidae (Gouramies) (Nelson 2006). An accessory respiratory organ, called labyrinth organ is a key character of gourami species. This organ is located next to the gill cavities and it is made up of folded membranes mounted on a bony frame. With the help of this organ these goumari species can use atmospheric oxygen directly and it enables them to live in poor oxygenated waters. Munshi and Hughes (1992) showed that gourami can still survive if air-breathing was prevented but it requires a higher amount of dissolved oxygen then. Similar statement was also stated by Ojha *et al.* (1977). According to Rahman (1989), three species belonging to the genus *Trichogaster* (*Colisa*) are found in Bangladesh. These are- *T. sota*, *T. fasciata* and *T. lalius*. However, last two species (Figures 1.1 and 1.2) have been selected for the present study.

**Figure 1.1:** The banded gourami, *Trichogaster fasciata* (male)

**Figure 1.2:** The dwarf gourami, *Trichogaster lalius* (male)

*T. fasciata* is commonly known as banded gourami or giant gourami or rainbow gourami or striped gourami; this species is locally called *khalisha*, *kolsa* and *khoila* in

Bangladesh. It is a declining species in Bangladesh and neighbor countries (e.g. India) and urgent conservation is suggested by the researchers in recent times (Mitra *et al.* 2007; Saha 2007; Chaki *et al.* 2014).

The other species, *T. lalius* is commonly known as dwarf gourami and locally called *lal khalisha*, *boicha* and *ranga khalisha*. It is also a rapidly declining species in Bangladesh and conservation measures are suggested by scientists (e.g. Saha 2007; Chaki *et al.* 2014).

## 1.2 Captive breeding

Simple breeding may be defined as the process of sexual reproduction and production of offspring of any species (Chowdhury 2007). Reproduction is essential for evaluating the commercial potentialities of its stock assessment, life history, culture practice and effective fishery management (Doha and Hye 1970; Biswas *et al.* 1984; Schaefer 1996 and 1998). Captive breeding is the propagation or preservation of animals outside their natural habitat involving control by humans of the animals chosen to constitute a population and of mating choices within the population (Chowdhury 2007). This breeding represents one of a myriad of tools conservation biologists have at their disposal to help prevent the extinction of a species, subspecies or population (Leus 2011). Captive breeding could be an effective tool to prevent a species from extinction. According to Gilpin and Soule (1986), the extinction process can roughly be divided in two phases. During the first phase, deterministic and often human caused threats such as habitat degradation and loss, direct exploitation of the species, competition from exotic and domestic species, killing due to human-animal conflicts etc., will cause the population(s) of a particular species or subspecies to decline. If these threats cannot be mitigated, this will eventually result in very small, fragmented and isolated remnant populations. The remnant populations are now so small that they become vulnerable to a number of other, non-human caused, threats and they enter phase 2 of the extinction process (Leus 2011). During this second phase of the extinction process, very intensive management of population and individuals, in addition to the in situ activities to mitigate any ongoing human caused threats, is often necessary to prevent extinction. Intensive management of populations and individuals can come in many different forms, for example translocation, breeding in a fenced area of wild habitat, supplementary feeding, captive hand rearing of young so that wild parents double clutch or become pregnant again sooner, captive breeding etc. Particularly in phase 2 of the extinction process, captive breeding can therefore be an important tool to help prevent extinction. It is however not the only tool and should also not be seen as a replacement for all other tools. It should rather been seen as one tool in a large tool box (Leus 2011).

Captive breeding initiatives should not merely produce larger numbers of individuals of a threatened species, they should also aim to maintain a high proportion of the gene diversity that is present in the wild population (Leus 2011). The gene diversity of a population represents the evolutionary potential of the population. It is only because not all individuals are genetically unique that natural selection can take place. Natural environments tend to be very variable and it is therefore important for the long term survival of the species that adaptation through natural selection can take place. Furthermore, higher levels of gene diversity tend to be correlated with higher fitness (Reed and Frankham 2003) and lower inbreeding levels (Leus 2011).

Using captive breeding to help save a species from extinction will only work if sufficient knowledge exists about the biological and husbandry needs of the species, so that sufficient numbers of offspring can be reliably bred from the desired parent combinations. This may require research and a sufficient number of individuals for the studies. However, if one waits to start a captive breeding program until the species is in imminent risk of extinction and little is known about the species' biology and husbandry, one often no longer has the time to await the results of studies and every individual is so important to the population that one cannot afford to run risks with research (Leus 2011).

However, some other reasons for captive breeding programs for species which are not (yet) in imminent danger of extinction are to use the individuals for conservation education and the raising of awareness (in the hope to contribute to reducing the human caused threats), and/or to use them as fundraising tools for other conservation activities etc.

According to Leus (2011), "the wild" is a very risky place compared to the relative safety of the captive environment. Reintroduction is also a relatively risky undertaking, especially in the beginning stages where many things have to be tried out for the first time and techniques and protocols have to be developed. If the captive population is the insurance population for the wild population, it would be unwise to extract individuals from this population before it has reached its target size and before the reproductive success is relatively predictable and fairly evenly spread over the population (*i.e.* one is confident that one can generally get most individuals to breed successfully and reproduction is not due to the chance reproduction of only a few individuals). Otherwise, one risks compromising the very population that is the insurance for survival of the species. In addition, the individuals to be reintroduced must be carefully chosen according to their genetic value to the captive population and to the wild population and according to the stages of the reintroduction process.

Research on captive breeding of aquatic fauna is seldom found in Bangladesh and it has recently been introduced in the country with a view to finding a solution to conserve the threatened and declining biodiversity. Hoq *et al.* (2014) carried out captive breeding program on mud crab, *Scylla serrata* in Cox's Bazar and succeeded. In another research Hossain *et al.* (2006) studied the breeding biology, captive breeding and fry nursing of humped featherback, *Notopterus chilata* in Jessore. However, there is a conspicuous need for more research efforts on captive breeding in Bangladesh.

## 1.3 Justification of the present study

In Bangladesh, gouramies are commercially important as both ornamental and food fish. They are medium sized fish belonging to the family Osphronemidae under the order Perciformes. The banded gourami (*T. fasciata*) and the dwarf gourami (*T. lalius*) are found in different rivers, beels, wetlands, and other freshwater bodies of Bangladesh (Saha and Hossain 2002; Ahmed *et al.* 2004; Amin *et al.* 2009, Galib *et al.* 2009a and 2013; Imteazzaman and Galib 2013; Mohsin *et al.* 2014; Chaki 2015; Fahad 2015; Joadder 2015). They are fascinating to observe and popular in the aquarium trade because of their brilliant colors (Das and Kalita 2006). The dwarf gourami, *T. lalius*, is the smallest of the genus and certainly one of the most beautiful and highly satisfactory and interesting ornamental fish; whereas *T. fasciata* is also a potential aquarium species that bred and adapts well to life in community aquaria (Talwar and Jhingran 1991).

Study of breeding of these two species can be very interesting because gouramies or anabantids of the genera *Colisa* and *Trichogaster* are well suited for reproductive behavioral study, being territorial and nesting species (Picciolo 1964). Both species are capable of breathing air through the accessory 'labyrinth organ' that is characteristic of gouramies and related species, and make occasional movements to the surface to gulp air (Das and Kalita 2006).

Gouramies have great demand in both national and international aquarium trade markets. In Bangladesh, ornamental gouramies are imported from foreign countries (Galib and Mohsin 2010) ignoring the potentiality of producing seeds locally. Gouramies are now being imported in many developed countries (e.g. United States, South Korea and other countries) from the south and south-east Asian countries including India, Thailand, and Singapore and so on (Axelrod and Vordwinkler 1995; Iwanowicz *et al*. 2001; Hossain 2008). So, sufficient production of these species can lead us to occupy foreign ornamental fish markets after fulfilling local demand.

Though gouramies are found in different water bodies of the country but seed production technology under captive conditions has not been developed yet and their reproduction is completely based on natural breeding in natural aquatic environments. In many of these water bodies of Bangladesh, status of gourami population is declining (Hossain *et al*. 2014; Mohsin *et al.* 2014) and now researchers are suggesting its conservation (Saha 2007; Chaki *et al.* 2014).

Thus, if it could be possible to develop the seed production technology under captive conditions, it would reduce, even stop the import of those fishes from foreign countries and it will help to develop both our economy and industry. There might be a possibility to export when seed production capacity would exceed the local demand.

Unlike land animals, aquatic animals such as fish and invertebrates produce large numbers of eggs, of which only a very few survive to adulthood as the next generation. There is a tradeoff between egg size and egg number for aquatic species, and two general types of "strategies" have evolved: one in which the number of eggs is very large and individual eggs are very small, and another in which the number of eggs is small but each egg is relatively large. Generally, the total mass of eggs a female can produce is limited by her size, so that a given egg mass comprises either many small eggs or few large ones (Weis 2003).

There are advantages to both of these strategies. The first strategy allows the potential number of offspring to be maximized through an increase in egg number (fecundity), whereas the second increases the likelihood that any given offspring will survive. The chances of survival are better for large eggs because fewer predators can consume large prey. The fecundity which is one of the most biological aspects of fish plays an important role to estimate the commercial potentialities of fish species concerned; it has direct relationship to fish reproduction and fisheries (Pathani 1981).

Understanding the early development of a fish is considered one of the most important steps for fish farmers (Kohinoor *et al.* 2003). The larger a fish egg is, the larger the larva it becomes, the fewer its possible predators, and the better its odds of survival; hence, large eggs lead to greater fitness. Natural selection favors those females that produce large eggs. Captive breeding provide opportunities to the target species to select mates naturally and would give better outputs. Breeding of any fish is affected by the environmental factors. Thus water quality parameters will be studied in this research. These factors regulate the reproductive cycle of a species of fish; round the year and also year from year (Viswanatha and Sundarar 1974).

To the best knowledge of the researcher, no such research effort on captive breeding of gouramies was carried out in Bangladesh. That is why the present study was undertaken by the researcher from June 2011 to conduct captive breeding of gouramies for the first time in Bangladesh where breeding programs were carried out using hormones (induced breeding) and by stocking them into waters of suitable breeding environment.

## 1.4 Research questions

The present study on captive breeding of two species of gouramies was carried out to answer the following research questions-

(1) Do the young collected from wild sources survive in captivity to take part in captive breeding trial?

(2) Do the studied species breed in captivity and what would be the breeding performance?

(3) Is it possible to produce seeds of studied species using inducing hormones?

(4) To what extent environmental parameters affect the breeding performances?

(5) What will be the survival status?

(6) What would be the market value of gouramies in local ornamental fish outlets?

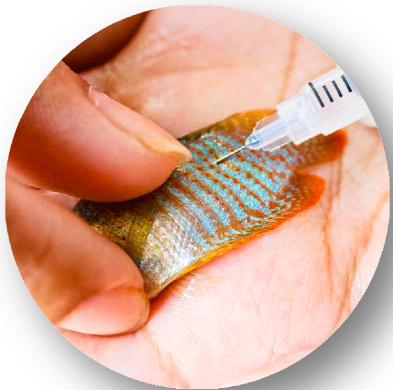
## 1.5 Specific objectives

The present study was designed with the following objectives-

1. to produce offspring of two gourami species in captivity;
2. to study related issues like length, weight, fecundity, breeding behavior and nursing of spawn;
3. to examine the larval development of gouramies in captivity;
4. to observe the survival status of newly hatched larvae;
5. to study water quality of breeding environment to understand the relationship with breeding and survival; and
6. to assess the ornamental fish market potentialities.

CHAPTER TWO

# REVIEW OF LITERATURE



# REVIEW OF LITERATURE

## 2.1 Overview

According to Nelson (2006) the genus *Trichogaster* is also known as blue gourami, is a member of anabantoid group of air-breathing fishes consist of about 120 species belonging to 19 genera and 3 families; found in freshwater habitats and indigenous to Africa and southern Asia. Froese and Pauly (2006) and Topfer and Schindler (2009) reviewed the relationship amongst the species of *Trichopodus* (formally *Trichogaster*) and it is a genus of tropical freshwater fish of gourami family found in Southeast Asia and gouramies of the *Trichopodus* genus are closely related to those of *Trichogaster* (formally *Colisa*). Again, Tan and Kottelat (2009) and Topfer and Schindler (2009) treated it under the genus *Trichogaster* and finally established the currently valid genus of Belontidae, which include *T. fasciata*, *T. labiosa, T. lalia* and *T. sota*.

The so-called labyrinth organ or accessory respiratory organ of *Trichogaster* and other anabantoid are of embryonic origin which is derived from the first and second gill arches and remain intimately associated with branchial structures in adults (Munsi 1976). Scientists (e.g. Graham 1997) have stated that a labyrinth organ, for assisting the exchange of gases, is present in all anabantoid species.

A range of researchers have worked on and described the morphology of *Trichogaster*. According to Pinter (1986) species belonging to this genus have shorter dorsal fin base and sexually mature individuals are much larger. The description, habitat mixing of species, breeding, diet and variants of gouramies in different parts of Africa, Asia and Europe were noted down and summarized by Goodwin (2001). Rainboth (1996) and Kottelat (2001) described the sexual dimorphism of gouramies and mentioned that the males of *Trichogaster* sp. can be promptly recognized by their dorsal fins with elongated posterior fin-rays which form a posterior pointed profile that may reach the caudal fin base.

Research findings have revealed that the habitats and distribution of *Trichogaster* are different parts of the globe, e.g. Singapore, Thailand, Cambodia, China, Myanmar, Vietnam, Malaysia, Sumatra, Bangladesh, India, Nepal and Pakistan (Hanitsch 1901; Tweedie 1953; Pinter 1986; Rahman 1989 and 2005; Shrestha 1994; Menon 1999; Mirza 2002; Oo 2002; Vidthayanon 2008). Paepke (2009) mentioned that species belonging to genus *Trichogaster* are tropical freshwater labyrinth fish of the gourami family found in Southeast Asia.

A number of researchers (Axelrod and Shaw 1967; Tooker and Miller 1980; Hails and Abdullah 1982; Hollis *et al.* 1997; Hollis 1999) have reported some complex reproductive behaviors of *Trichogaster* species, especially of male individuals. The maturation of *Trichogaster* species has been focused in a number of research efforts by Lee and Ingersoll (1978), Hails and Abdullah (1982), Becker *et al.* (1992), Degani and Boker (1992a and 1992b), and Jackson *et al.* (1994).

Degani (1989) stated that male of gourami will incorporate bits of plants, twigs and other debris, which hold the nest together better. Several scientists focused on the researches regarding health issues, especially disease. Yooyen *et al.* (2006) investigated on the helminthic (parasite) infections in freshwater gourami from the Bung Borapet Reservoir of Thailand. Gouramies can thrive in environments with not only widely different substances ranging from sand to large rock, but also widely varying water quality parameters (Geisler *et al.* 1979).

Gouramies are popularly used as food fish in Bangladesh, especially in rural areas of the country. Species like *T. fasciata* and *T. lalius* were reported to be consumed not only in fresh condition but also as sun-dried form in Bangladesh (Samad *et al.* 2009 and 2010).

## 2.2 Species profile

Among the three gourami species of Bangladesh (Rahman 1989), two species, *T. fasciata* and *T. lalius* have been selected for the present study. A short description of these two species are described here-

### 2.2.1 Banded gourami, *Trichogaster fasciata* Bloch and Schneider 1801

***Classification*** (Nelson 2006)

Phylum: Chordata
  Class: Actinopterygii (Ray-finned fishes)
    Order: Perciformes (Perches)
      Suborder: Anabantoidei
        Family: Osphronemidae (Gouramies)
          Subfamily: Luciocephalinae
            Genus: *Trichogaster*
              Species: *T. fasciata*

***Synonyms***

*Colisa fasciata* Bloch and Schneider 1801
*Colisa fasciatus* (Bloch and Schneider 1801)
*Polyacanthus fasciatus* (Bloch and Schneider 1801)
*Trichopodus colisa* Hamilton 1822
*Trichopodus bejeus* Hamilton 1822
*Trichopodus cotra* Hamilton 1822
*Colisa vulgaris* Cuvier 1831
*Colisa ponticeriana* Valenciennes 1831

***Common/local names***

English: Banded gourami, giant gourami, rainbow gourami and striped gourami (Froese and Pauly 2006).

Bangladesh: Khalisha, Kolsa and Khoila (Bhuiyan 1964; Saha 2007; Rahman 1989 and 2005)

**Geographical distributions:** Bangladesh, India, Nepal, Pakistan and Upper Myanmar (Talwar and Jhingran 1991; Saha 2007).

**Conservation status:** Not threatened in Bangladesh (IUCN Bangladesh 2000). But Saha (2007) mentioned that this species need to be conserved. Chaki *et al.* (2014) also emphasized the conservation need for gouramies. Chakraborty *et al.* (2012a) ranked this species as Vulnerable in 2008 to 2009 and as Endangered in 2010 in the Meduary Beel in Mymensingh district of Bangladesh.

**Morphology:** Strongly compressed egg-shaped body with small mouth. Upper is lip thick and papillose, especially in old males (Talwar and Jhingran 1991). Pelvic fins thread-like and caudal truncate. Bases of dorsal and anal fins are broad. Lateral line scale 29-31 (Bhuiyan 1964; Shafi and Quddus 2001). Body color greenish with orange or bluish bars descending obliquely downwards and backwards from back to anal fin. Vertical fins are with alternate dark and pale spots or bars; anal fin often with red margin (Talwar and Jhingran 1991).

Fin formula:

D. 15-17/9-13; A. 15-18/14-19; P. 10; V. I, V. 16 (Bhuiyan 1964)

D. XV/10-14; $P_1$. 9-10; $P_2$. 1; A. XV-XVIII/15-19; C. 15-16 (Rahman 1989 and 2005; Saha 2007)

D. XV-XVII 9-14; A XV-XVIII 14-19; P. 9-10 (Talwar and Jhingran 1991)

D. XV-XVII/9-13; P. 10; V. 1; A. XV-XVIII/14-19; C. 16 (Shafi and Quddus 2001)

**Maximum lengths:** 12 cm (Talwar and Jhingran 1991), 8 cm (Bhuiyan 1964), 10 cm (Rahman 1989 and 2005), 12.7 cm (Shafi and Quddus 2001).

**Habitats:** Widely distributed in different types of water bodies of Bangladesh (Rahman 1989 and 2005; IUCN Bangladesh 2000; Saha 2007). This species inhibits large rivers and estuaries, also tanks, ditches and ponds (Talwar and Jhingran 1991). Live in Beels, Baors (Oxbow lakes), ponds and rivers (Shafi and Quddus 2001). This species was recorded in the various water bodies of Bangladesh- the Andharmanik River (Mohsin *et al.* 2014), Atrai River (Chaki *et al.* 2014), Halti Beel (Imteazzaman and Galib 2013), Chalan Beel (Galib *et al.* 2009a and 2010), Choto Jamuna River (Galib *et al.* 2013b), Jamuna River (Shahjahan *et al.* 2001), Padma River (Mohsin *et al.* 2013; Joadder *et al.* 2015) and Ramnabad River (Ali *et al.* 2015). This species prefers clear water (Shafi and Quddus 2001).

**Food and feeding:** This is an omnivorous fish species and can feeds on 150 larvae and larvae a day (Ahmad 1943; Shafi and Quddus 2001; Saha 2007; Zaman *et al.* 2012). Analysis of ingested food contents has revealed that the food contents of this species are as follows, algae 51%, aquatic vegetation 25%, protozoa 15%, mud and sand 9% (Bhuiyan 1964; Shafi and Quddus 2001). It occupies the third trophic level in the food chain in the aquatic ecosystem (Saha 2007).

**Breeding:** *C. fasciata* breeds in the paddy and jute fields during rainy season. Its forms bubble nest during this time and guards the next carefully. Eggs are yellowish and non-adhesive (Saha 2007). Diameter of the eggs is 1.0-1.15 mm and hatch between 14 and 16 hours at 84-92 °F. Yolk sac exhausted after 72 hours of hatching (Shafi and Quddus 2001).

**Fishery info:** Usually harvested by cast net, khalisa net, veshal net and hocha (Shafi and Quddus 2001); gill net, lift net, push net, fixed net and seine net (Galib *et al.* 2009b). It is traditionally liked for its good taste in India; also it is easily bred and adapts well to life in community aquaria (Talwar and Jhingran 1991). It can be cultured in shallow seasonal ditches, rice fields, shallow flood plains, extended shallow areas of beels (Saha 2007). This species is highly esteemed as food fish in Bangladesh (Bhuiyan 1964) and liked by all for its good taste and because it can be eaten bones and all (Saha 2007). Both fresh and dried forms are popular for consumption (Samad *et al.* 2009 and 2010).

### 2.2.2 Dwarf gourami, *Trichogaster lalius* (Hamilton 1822)

***Classification*** (Nelson 2006)

Phylum: Chordata
- Class: Actinopterygii (Ray-finned fishes)
  - Order: Perciformes (Perches)
    - Suborder: Anabantoidei
      - Family: Osphronemidae (Gouramies)
        - Subfamily: Luciocephalinae
          - Genus: *Trichogaster*
            - Species: *T. lalius*

***Synonyms***

*Colisa lalia* (Hamilton 1822)

*Trichopodus lalius* Hamilton 1822

*Colisa lalius* (Hamilton 1822)

*Colisa unicolor* Cuvier 1831

***Common/local names***

English: Dwarf Gourami (Froese and Pauly 2006)

Bangladesh: Lal Khalisha, Boicha and Ranga Khalisha (Saha 2007; Rahman 1989 and 2005)

**Geographical distributions:** Bangladesh, India (Ganga and Yamuna river systems), Pakistan and Borneo (Talwar and Jhingran 1991). In Bangladesh it is available in the Ganges and the Jamuna River systems (Wahab 2007).

**Conservation status:** Not threatened in Bangladesh (IUCN Bangladesh 2000). But Chaki *et al.* (2014) emphasized the conservation need for gouramies. Chakraborty *et al.* (2012a) ranked this species as Lower Risk in 2008, Vulnerable in 2009 and Endangered in 2010 in the Meduary Beel in Mymensingh district of Bangladesh.

**Morphology:** Strongly compressed egg-shaped body with small mouth. The dorsal and anal fins are soft and rounded. Lateral line incomplete and interrupted (Rahman 1989). 27 or 28 large scales are in longitudinal series. Anal fin densely scaled. Body scarlet, crossed by somewhat oblique bands of pale blue. Fins with scarlet spots or bars; anal fin with red margin (Talwar and Jhingran 1991).

Fin formula:

D. XV-XVII/7-8; $P_1$. 8-9; $P_2$. 1; A. XVII-XX/14-16 (Rahman 1989 and 2005)

D. XV-XVII 7-10; A. XVII-XVIII 13-17; P. 10 (Talwar and Jhingran 1991)

D. XV-XVII/7-10; $P_1$. 8-10; $P_2$. 1; A. XVII-XX/13-17 (Wahab 2007)

**Maximum lengths:** 8.8 cm (Rahman 1989 and 2005), 5 cm (Talwar and Jhingran 1991).

**Habitats:** Widely distributed in different types of water bodies of Bangladesh (IUCN Bangladesh 2000). This species was recorded in the various water bodies of Bangladesh- the Andharmanik River (Mohsin *et al.* 2014), Atrai River (Chaki *et al.* 2014), Halti Beel (Imteazzaman and Galib 2013), Chalan Beel (Galib *et al.* 2009a), Choto Jamuna River (Galib *et al.* 2013b), Padma River (Mohsin *et al.* 2013; Joadder *et al.* 2015) and Ramnabad River (Ali *et al.* 2015).

**Food and feeding:** In nature this species eats small insects and larvae from the surface of water, and grazes algal growth on plant (Wahab 2007).

**Breeding:** In breeding season, the male courts the female with mild aggression and then builds a bubble nest among plants. Male also guards the nest and surrounding areas of territory (Wahab 2007). A female may produce about 600 eggs (Riehl and Baensch 1991). After 12-24 hours of laying eggs, they hatch and the fry become free-swimming after three days (Wahab 2007).

**Fishery info:** Usually harvested by cast net, gill net, lift net, push net, fixed net and seine net (Galib *et al.* 2009b). This fish often sold alive at Calcutta and they thrive well in an aquarium (Talwar and Jhingran 1991). Used as food fish in Bangladesh, in both fresh and dried forms (Samad *et al.* 2009 and 2010).

## 2.3 Captive breeding and relevant issues

To the best knowledge of the researcher, no previous studies were carried out on breeding of *Trichogaster*/*Colisa fasciata* and *C. lalius* in Bangladesh so it is not possible to review literature on mentioned subject. However few other studies on other aspects like length-weight relationship, condition factors of these species were found to be conducted outside Bangladesh. In this chapter available literatures relevant to the present study will be reviewed. In addition to this issue, captive breeding programs carried out for other aquatic vertebrates will also be reviewed in this section of thesis.

### 2.3.1 Studies on gouramies

**Abujam *et al.*** (2015) carried out captive breeding of giant gourami, *T. fasciata* in India. The mature *T. fasciata*, the length ranged from 7.3-9.5 cm and weight from 9.94-12.9 g (females) while the length varied from 7.5-9.5 cm and weight from 9.35-14.85 g (males) were selected for acclimatization and formation of bubble nest. Later, 1 female and 2 male were injected with different doses of ovaprim hormone which was ranged from 0.3- 1.0 ml $kg^{-1}$ and released in the three breeding aquariums and one aquarium was kept as control. In each breeding trial, the same doses of ovaprim were injected in both the sexes and breeding trials was repeated 3 times. The latency period was observed from 9 to 15 hours, the numbers of egg ranged from 190 to 429, percentage of fertilization was recorded from 55.28 to 71.53, the fertilized eggs varied from 111 to 289, percentage of hatching was found between 29.25 and 45.54 and number of hatching ranged from 56 to 184. Based on the breeding trials, it could be predict that the first trial was most effective one (0.3 to 0.5 ml $kg^{-1}$). Hence, this doses was reasonably suitable for breeding program and followed by second trial (0.5 to 0.7 ml $kg^{-1}$) which is slightly effective than the third breeding trial (0.8 to 1.0 ml $kg^{-1}$). The research findings revealed that the lower doses of ovaprim was effective than the higher and can be induced for best breeding program.

**Motilan and Nishikanta** (2015) studied the captive breeding of thick-lipped gourami, *Trichogaster labiosua* (Day) by gradual increasing aquarium water temperature and their early life stages. In their research, for the breeding of *T. labiosua*, male and female individuals were kept in the ratio of 1:1 in clean and clear water containing the aquatic weeds, *Hydrilla* sp. *T. labiosua* easily bred in the aquarium conditions on gradually increasing temperature of aquarium water. Nesting behavior of the fish was observed within the temperature range 24 to 28 $^{0}$C. Spawning rate ranged from 20,000−25,000. The developmental period was observed between 18 and 25 days.

**Hossen *et al.*** (2014) embryonic and larval development of striped or banded gourami, *Trichogaster fasciata* at the Bangladesh Agricultural University Campus of Bangladesh. The researchers have found that the fertilized eggs were spherical, transparent, buoyant, non adhesives and brownish in color, with an average diameter of 0.30-0.60 mm. First cleavage occurred within 25-30 min of postfertilization at 26±1°C. Hatching started at 22 h post-fertilization and completed within 24 h at the same temperature range. New hatchlings were 2.0-2.5 mm long devoid of mouth and pigmentation and started feeding within 48-60 h post-hatching. Depletion of the yolk-sac and development of the gills occurred on the second day of hatching.

***Paswan et al.*** (2012a and 2012b) studied length-weight relationship (LWR) of *Trichogaster (Colisa)* species belongs to the Belontidae family inhabiting

Brahmaputra basin, were studied. The fishes *T. fasciata* and *T. sota* were obtained from fish landing site at different floodplain beels of Brahmaputra basin during 2010 to 2011. The parameters 'a' and 'b' of the length-weight relationship of the form W = aLb and condition factor (K) = Wx1000/$L^3$ are presented for the two fish species. The results reveals that the value of regression coefficient 'b' of *T. fasciata*, tends to be higher during pre-monsoon and lower during winter while in *T. sota*, it tends to be higher during winter and lower during post-monsoon seasons. The exponential index 'b' for female (2.040) was found to be lower than that of male (2.424) in *Trichogaster* species while male (1.042) was lower than female (1.232) in *T. sota*. The 'K' value was found between 16.87 and 22.19 for *T. fasciata*, while it was between 19.71 and 33.59 for *T. sota*. This indicates an allometric relationship in the growth and healthy condition for both the species.

**Huang *et al.*** (2008 and 2010) studied and reported the morphological and functional expressions of two anabantoid fish species.

**Mitra *et al.*** (2007) studied the biology and fishery of banded gourami, *Colisa fasciata* in a floodplain wetland of Ganga River basin, India. They have recorded that the breeding period of this species was March to September. The regression model for length and weight of the fish was also established by the researchers. They also found that the catch was maximum during October-February, the post spawning period, and lower during March-September, the breeding period.

**Saha** (2007) mentioned that *C. fasciata* breeds several times in the stagnant water of paddy fields during rainy season. At this time of spawning, male embraces the female. Eggs are yellow in color and non-adhesive, and kept in the floating nest.

**Das** (2003) described different strategies for captive breeding of ornamental fish species in India and mentioned that species belonging to genus *Trichogaster* (*Colisa*) are known to be breed in a little weed infested water tanks.

**Sanford** (1999) described that the breeders of dwarf gourami, *T. lalia* have created different color variations, varying degree of red or blue coloring.

The genetic study including inheritance of coloration and banding patterns of *Trichogaster* and some other fishes have been studied by **Frankel** (1992, 1997, 1998, 2005 and 2008).

**Pollak *et al.*** (1978 and 1981) studied the spawning and maturity of *Trichogaster* species. Correlation between gonadotropin and sexual behavior of male blue gourami was studied by **Mananos *et al.*** (1997).

**Bhuiyan** (1964), **and Shafi and Quddus** (2001) mentioned that *Trichogaster* (*Colisa*) *fasciata* breeds in the paddy and jute fields during rainy season. Its forms bubble nest during this time and guards the next carefully. Eggs are yellowish and non-adhesive. Diameter of the eggs is 1.0-1.15 mm and hatch between 14 and 16 hours at 84-92 °F. Yolk sac exhausted after 72 hours of hatching.

**Piccolo** (1964) studied the sexual and nest discrimination behavior of the genera *Colisa* and *Trichogaster* and describes these species as territorial and nesting. He also mentioned that territorial males have a higher differentiated behavior towards males and females of the same species. These fishes are sexually dimorphic and display marked changes in coloration that are associated with the various parts of the reproductive cycle. At the onset of the spawning cycle, a male constructs a bubble nest at the air-water surface with bubble and usually with a bit of vegetation.

**Hanitsch** (1912) described that controlling of mosquito is possible as *Trichogaster* species take mosquito larvae as their food in nature. Several workers have been carried out researches and reported on the reproductive biology of *Trichogaster* species from outside Bangladesh. A number of works have been carried out on the reproductive biology of *Trichogaster* species, especially *T. trichopterus*, blue gourami. Research findings shown that the fecundity of this species varies with the size of female and eggs varied from 300 to 400, sometimes up to 1000 for smaller individuals and between 2000 and 4000 from larger individuals (**Zukal** 1983; **Richter** 1988; **Pethiyagoda** 1991).

### 2.3.2 Captive breeding in Bangladesh

**Afroz *et al.*** (2014) carried out captive breeding of *Mastacembelus pancalus* at the Jessore University of Science and Technology, Jessore and found that the larvae survive up to 390 hours of hatching and improved survival rate showed in rain water than pond and supplied tap water. The researchers have categorized the larval development progress into nine distinguish stages. In the first stage, larvae carried yolk sac on their body. In later stage, yolk sac was convex interiorly and become tubular. Yolk sac was completely disappeared in the third stage within 80-90 hours of hatching and started to exogenous feeding. Eyes were fully pigmented at the fourth stage. In fifth stage, intestine and kidney were developed; whereas, well-developed jaws were visualized in the sixth stage. In later stages, gut and airbladder were visualized. In the eighth stage, distinct anal and dorsal fin were observed. At the last stage, spiky notochord was shown within 370-390 hours of hatching but yet to complete larval stages.

**Hasan** (2014) observed and described the captive breeding and seed production techniques of endangered giant river catfish, *Sperata seenghala*, locally known as "guizza air''. The captive breeding was carried out at Desh Bondhu Hatchery of Mymensingh from April 2014 to August 2014. A total of 60 pairs of adult *S. seenghala* (850 to 1500 g) were collected from the Brahmaputra river-basin and floodplains of Mymensingh region and stocked in a 40 decimal pond with 2.5-3 feet depth three months before the onset of the breeding season. In view of stimulating natural propagation of *S. seenghala*, the spawning pond was supplied with fresh groundwater to maintain a natural condition and fertilized with cow dung, urea and TSP at a rate of 5 kg, 200 g and 200 g per decimal, respectively. At the onset of breeding, *S. seenghala* excavated holes (nests) of about 1.5-2 feet diameter with 6-7 inch depth at the edge of the pond dike at about 2-2.5 feet below the water surface where female laid eggs at night during 4th week of June, 2014. Following spawning, females protected their nests and showed extreme parental care. Near the end of and immediately after the yolk sack absorption females fed their young with their own skin mucus. Fry numbering 50,280 with a mean length and weight of 1.91±0.24 cm and 0.25±0.07 g, respectively were collected by repeated netting with mosquito net from the spawning pond after 15 days of spawning. The final average length and weight of the fingerlings were 6.26±0.21 cm and 2.55±0.08 g, respectively after four weeks of nursing. The survival rate of fry was 63%.

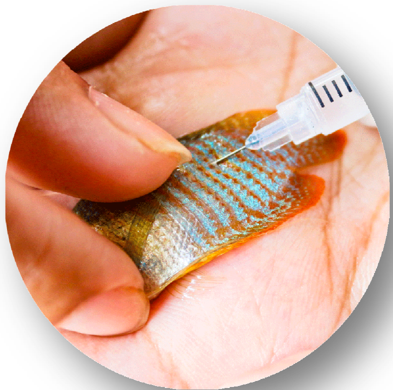
**Hoq *et al.*** (2014) carried out captive breeding program on mud crab, *Scylla serrata* in Cox's Bazar and succeeded. In their research, fully gravid mud crabs, *Scylla serrata* (230 to 360 g body weight; 7.5 to 10.25 cm carapace length) were bought from tidal floodplain of Mosehkhali, Cox's Bazar and transported to the Niribili Hatchery, Krjukhal, Ukhia, Cox's Bazar. The crabs were housed in cemented cistern, equipped with treated sea water supply. They were held in 10 tons cemented tanks provided with sand as substrate and PVC pipe for shelter. After 10-15 days of acclimation, unilateral eyestalk ablation of berried crab was applied to induce spawning. One to two days prior to hatching, the gravid mud crabs were moved to the laboratory of Marine Fisheries & Technology Station, Cox's Bazar and kept in 300-L fiber glass tanks with central aeration system. The laboratory was maintained with proper bio-security and aeration system, enough lightening and pretreated sea water supply facilities. After 30-32 days of eyestalk ablation, egg formation was started and the crabs finally spawned after 45-47 days of eyestalk ablation. The hatching process was completed within two days in fiber glass tanks. Aeration in the hatching tank was therefore turned off for several minutes and the larvae (zoea-I) that were actively swimming up to the surface were collected by gently scooping them from the surface.

**Hossain *et al.*** (2006) studied the breeding biology, captive breeding and fry nursing of humped featherback, *Notopterus chilata* in Jessore during 1998 to 2001. They have found that the peak period of ovulation of the study species was from July to August. The researchers have also mentioned that *N. chitala* attained its sexual maturity at third year of age and captive breeding was most effective in cemented breeding tanks. They also noted that the highest survival was found when fed with *Barbodes gonionotus* fry and *Tubifex* sp.

Captive breeding of *Trichogaster lalius* (Hamilton, 1822) and *Trichogaster fasciata* Bloch and Schneider, 1801

CHAPTER THREE

# METHODOLOGY



# METHODOLOGY

Descriptive methodology followed in carrying out the present study is presented here under different headings-

## 3.1 Study area and duration

The present study was conducted in the experimental ponds and the Aquatic Biodiversity Lab of the Department of Fisheries, University of Rajshahi, Rajshahi, Bangladesh. The experimental site is located at approximately 24°22' North and 88°38' East (Figure 3.1). Experiments were conducted in both small ponds and aquaria. This study was conducted for a period of four years from July 2011 to June 2015. A brief description of different activities performed in these years is shown in Table 3.1.

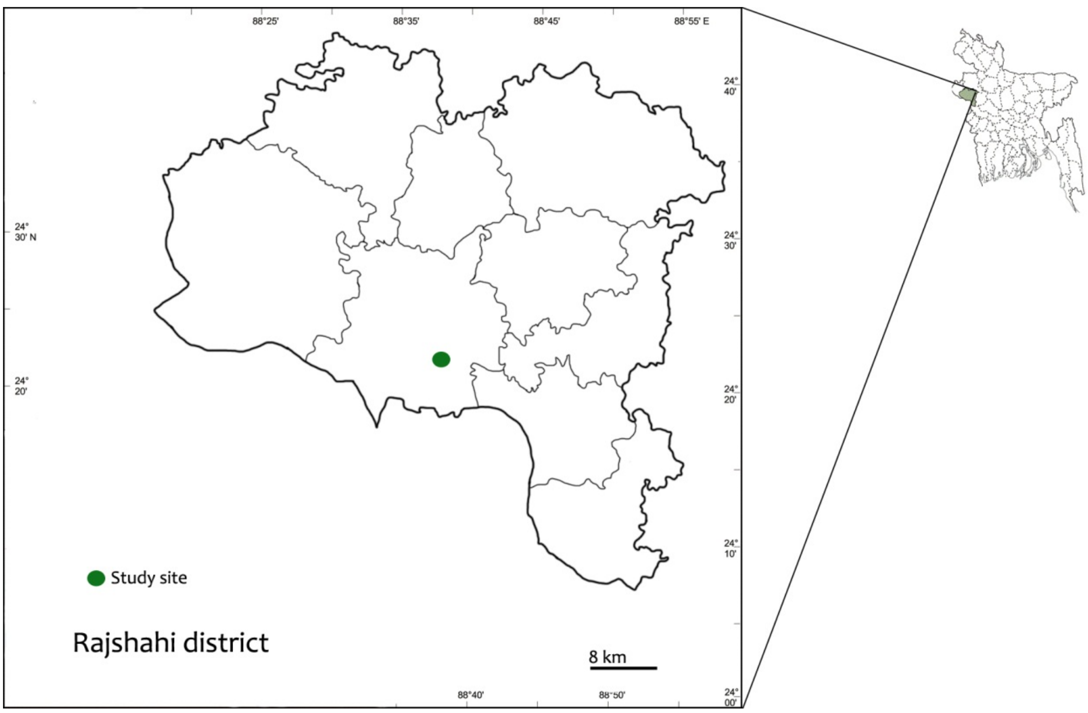



**Figure 3.1:** Map of Rajshahi district showing the study site.

**Table 3.1:** Research activities in different years

| *Year* | *Major activities performed* |
|---|---|
| 2011-2012 | Collection of juvenile or brood from wild, domestication; review of literature |
| 2012-2013 | Breeding trial, estimation of fecundity and water quality analyses |
| 2013-2014 | Breeding trial, and water quality analyses |
| 2014-2015 | Statistical analyses of data; thesis writing |

## 3.2 Collection and transportation of juvenile or brood fishes

At the very beginning of the study juvenile or brood fishes were collected from the wild sources of Rajshahi and adjacent districts, primarily from the Chalan Beel, the Halti Beel and the river Padma (Figure 3.2). The studied species were not commonly found in all parts of these three water bodies. A preliminary survey was carried out in which local fishermen were asked to get an idea of availability of studied species. Then fishing nets (push net, *Thela Jal*; and lift net, *Khora Jal*) and fishing traps (locally called *Kholsun* and *Dohair*) were employed to collect young or brood fish in areas recommended by the fishermen. In addition to these fishing gears, individuals were also collected through hand fishing method. Mesh size of the fishing nets used in the collection of fishes was varied between 1-3 cm. Thus, small or fry of targeted and other species could easily be escaped.


**Figure 3.2:** Map showing the brood collection spots

Transportation of the collected individuals was a big challenge in this study. The two collection sites (Atrai River and Chalan Beel) were far from the experimental site, Aquatic Biodiversity Lab of the Department of Fisheries, University of Rajshahi. However, about 300 young and mature individuals of both the studied species were transported from those two sampling sites in an aluminum container. Though gouramies are air-breathing fishes but transportation was really crucial because of longer duration of transportation and comparatively higher temperature of the greater Rajshahi region. During transportation, veterinary saline was added to water in the container at standard dose.

## 3.3 Rearing and domestication of juvenile or brood fish

Collected Brood were transported to the laboratory and reared in rectangular glass aquaria with artificial diet (Figure 3.3). Male and female individuals were reared separately in different aquaria. These aquaria were provided with approximately 5 cm thick bottom mud and live aquatic vegetation, especially water velvet (*Pistia* sp.) and water hyacinth (*Eichhornia* sp.).

At the time of rearing in aquaria, the individuals were fed with commercial aquarium feeds twice a day. The first feeding was done in the morning around 8 am and at night around 8 pm local time (GMT +6.00 hours). However, boiled egg yolk and live feed (*Tubifex* sp.) were also provided thrice and twice a week respectively. Length and weight of the reared fishes were recorded at regular interval by using an electronic balance and slide calipers.

**Figure 3.3:** Rearing of *Trichogaster fasciata* brood (male) in an aquarium

## 3.4 Estimation of fecundity

Fish fecundity is associated with studies of natural mortality, racial characters and total population estimation (Rath 2000). For the estimation of fecundity, visually matured females were dissected and ovaries were taken out. Then the ovaries were weighed; three sub-samples were taken from the front (anterior), mid (middle) and rear (posterior) sections of each ovary and weighed. Then the total number of eggs in each ovary sub sample was proportionally estimated using the following equation,

$$\text{Fecundity} = \frac{Gonad\ weight\ X\ number of eggs\ in\ the\ subsample}{Subsample\ weight}$$ (Yelden and Avsar 2000)

Later, by taking the mean number of three sub-sample fecundities ($F_1$, $F_2$, and $F_3$), the total (absolute) fecundity for each female fish was estimated using the following formula,

$$F_T = \frac{F_1 + F_2 + F_3}{3}$$ (Yelden and Avsar 2000)

Where, $F_T$ is the total or absolute fecundity, and $F_1$-$F_3$ are the number of eggs in the sub-samples 1, 2, and 3 of gonad (ovary). A total of thirty individuals of each species were studied to find out the fecundity of gouramies.

## 3.5 Preparation of breeding ponds and aquaria

One month before stocking into the experimental ponds for the purpose of breeding, the ponds were cleaned and prepared purposively. Existing aquatic vegetations were removed manually with the help of simple devices (Figure 3.4). After eradicating aquatic vegetations, chemical poison was applied into the experimental ponds to remove undesired fish and other aquatic animals from the ponds. Then the ponds were left for ten days to ensure the disappearance of harmful effects of poison.

Immediately after poisoning, the ponds were fenced by mosquito nets (Figure 3.5). The purpose of providing mosquito nets was to create a barrier or fence to the unwanted animals living outside such as snakes, frogs and so on from entering into the ponds. Mosquito nets were installed in such a way that outsider animals cannot evade the fence. Aquatic vegetation was allowed to grow again for creating a suitable environment for natural breeding.

**Figure 3.4:** Aquatic vegetation removal during preparation of pond

**Figure 3.5:** Fencing by mosquito net for creating barrier to unwanted animals

The breeding aquaria were also cleaned and dried under the direct sunlight prior to carrying out captive breeding programs. After proper drying, the glass aquaria were placed on appropriate racks or frame and filled with supplied water. Each aquarium was provided with river sand and small gravels with smooth surface and the thickness of bottom bed was 3 cm to make a favorable environment for the aquatic vegetations and also for the captive breeding. Aquatic vegetations placed in the experimental aquaria were collected from nearby ponds and washed thoroughly, before placing them into the aquarium, by clean water to remove any unwanted organisms (e.g. snail) or debris attached to the leaves of plants (Figure 3.6). Aquaria were set on the near the balcony where direct sunlight was available for approximately 5-6 hours a day. The vegetations were allowed to attach efficiently and for this purpose breeding pairs were stocked at least seven days after plantation.

**Figure 3.6:** Experimental aquarium with floating aquatic vegetations

## 3.6 Transportation and release of brood

Selected broods for the breeding program were transported to experimental ponds using plastic bags (Figure 3.7). After placing them into the plastic bags, the bags were taken into another suitable bag to ensure safety from direct sunlight so that water temperature could not be increased to an extent that is beyond the tolerance level for the fishes. These broods were released in the afternoon in order to avoid direct sunlight and it allowed the brood to adopt themselves easily with the new environment (Figure 3.8).

**Figure 3.7:** Carrying of brood into the plastic bags

Before releasing the brood, the plastic bags with brood were kept floated into the water for 30 minutes for acclimatization to minimize the differences in water temperatures.

**Figure 3.8:** Release of brood gouramies into the pond

## 3.7 Sexual dimorphism

Gouramies are sexually dimorphic and display marked changes in coloration that are associated with the various parts of the reproductive cycle. Conspicuous differences in morphology between male and female gouramies are visible, especially during the time of breeding or when they reached at sexual maturity. Sexual dimorphic characters of gouramies are presented in Table 3.2, Figures 3.9 and 3.10.

**Table 3.2:** Sexual dimorphic characteristics in studied species

| ***Species/Issues*** | ***Male*** | ***Female*** |
|---|---|---|
| Giant gourami, *Trichogaster fasciata* | | |
| Coloration | More | Less |
| Vertical bands on body | Peacock blue | Normal |
| Pectoral fins | Orange | Not orange |
| Outer margin of anal fin | Peacock blue | Normal |
| Abdomen | Somewhat slender | Enlarged |
| Size at same age | Larger | Smaller |
| Genital papilla | Not so prominent | Prominent bulge |
| Dwarf gourami, *Trichogaster lalius* | | |
| Coloration | More, brighter | Less/No coloration |
| Outer margin of fins | Reddish or orange | No such coloration |
| Abdomen | Somewhat slender | Enlarged |
| Size at same age | Larger | Smaller |
| Genital papilla | Not so prominent | Prominent bulge |

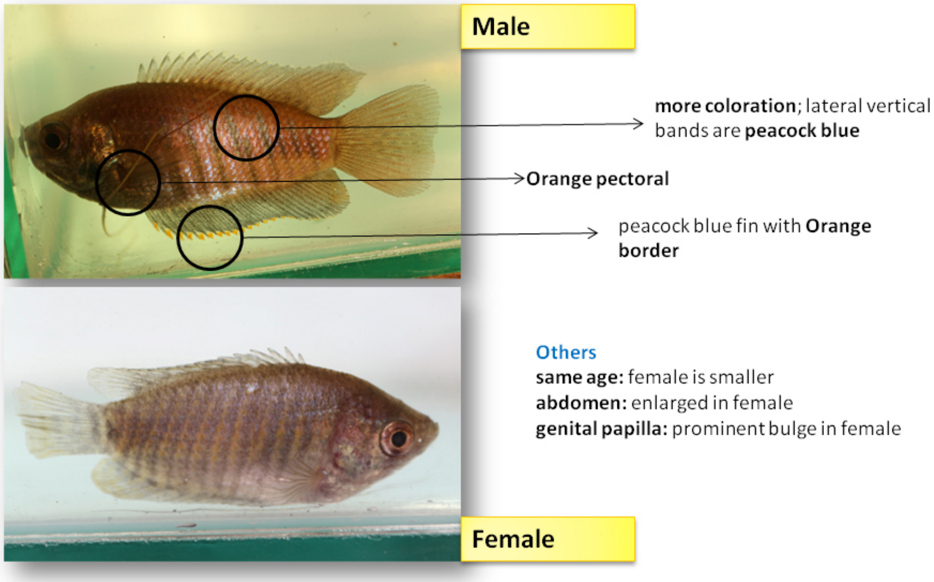



**Figure 3.9:** Sexual dimorphic characters in giant gourami, *Trichogaster fasciata*

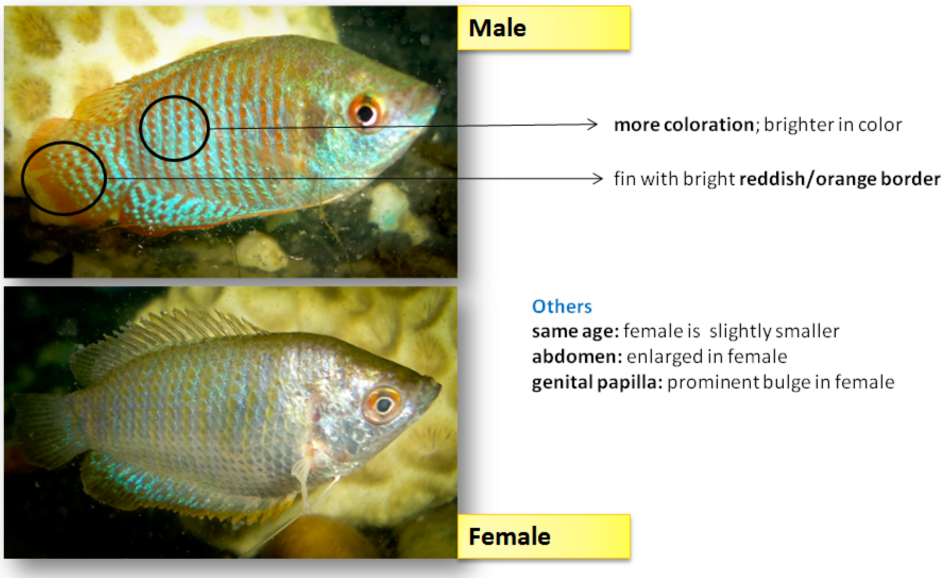



**Figure 3.10:** Sexual dimorphic characters in dwarf gourami, *Trichogaster lalius*

## 3.8 Breeding plans

### 3.8.1 Selection of hormones and doses

Breeding plan was made based on the breeding season of concerned species, during rainy season especially from the month of May to September. After collecting and rearing the brood fishes some of them were subjected to hormone administration.

Two hormones, Pituitary Gland (PG) and *Ovulin* (synthetic hormone) were used in this research (Figure 3.11).

*Ovulin*, a synthetic hormone, is probably the most popular synthetic hormone in Bangladesh in recent time. The synthetic hormones are being introduced lately in artificial breeding of finfishes in the hatcheries of Bangladesh which stimulate gonadotropin release and ovulation in teleosts. Each 10 ml of this hormone contains Dom 100 mg and S-GnRH 200 ug. It's a liquid peptide supplement that is used to compress the spawning season, coordinate the spawning time and increase the milt production in males. *Ovulin* is used in all teleost fish including salmon, Arfican catfish, perch, per-like fish and carp fish. *Ovulin* contain an analogue of S-GnRH and a brain neurotransmitter (dopamin) inhibitor. The GnRH in ovulin elicits the release of stored gonadotropins from pituitary. The Dopamine inhibitor serves to remove other inhibitor of GnRH release.

Application of PG in the induced breeding of fishes in hatcheries of Bangladesh is most commonly found. In the present study, PG was also used as inducing agent for breeding of gouramies. Fresh PGs were collected from the commercial vendors of Chachra area of Jessore district.

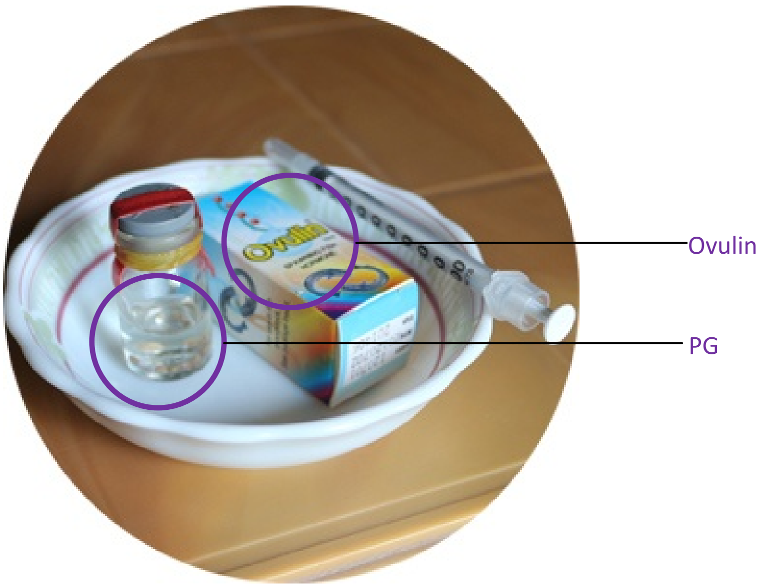



**Figure 3.11:** Hormones used in the research

In the beginning of this study, the hormones were administered at different doses to assess the performance and to select a standard dose of hormones for the present research. PG was injected at 2 mg/kg, 5 mg/kg and 10 mg/kg and after the trial it was found that performances of 5 mg/kg (female) and 2 mg/kg (male) were positive.

Thus this combination of dose was selected for PG for the present research. In the same manner *Ovulin* was also injected at 0.5 ml/kg, 1 ml/kg and 2 ml/kg and it was found that individuals, both male and female, breed at 0.5 ml/kg. So, this dose was selected for the concerned hormone category.

### 3.8.2 Injecting hormones

As the studied species were smaller in size, in comparison to other species which are subjected to hormone administration in hatcheries of Bangladesh, intra-muscular injections (Figure 3.12) were given instead of intra-peritoneal injection. Standard needle and syringe were used for this purpose. However, after injection the injection sites breed for sometimes but it did not kill any fish.

**Figure 3.12:** Intra-muscular hormone administration in dwarf gourami, male (left) and female (right)

### 3.8.3 Experimental design

In this research the size of each experimental earthen pond was 0.75 decimal (0.0030 ha) and the size of each aquarium was 60x35x35 cm (length x width x height). There were three treatments ($T_1$, $T_2$ and $T_3$) in the present study each with three replications (Table 3.3). The first treatment ($T_1$) was set with a view to assessing the breeding performance of studied species in natural conditions, without the use of any inducing hormones. This treatment also treated as control in the present study. In other two treatments, mature individuals of the gourami species were subjected to PG and synthetic hormone administration.

Breeding of studied species in captivity was carried out for two seasons, firstly from June to August of 2013 and secondly from the same time duration in 2014. In aquaria, one gravid female and two males were stocked for the purpose of breeding.

Three (3) pairs of mature brood of each species were stocked into three different experimental ponds. Both environments were provided with aquatic vegetation to stimulate the natural breeding of stocked species. An outline of the total breeding plans is shown in Table 3.3.

**Table 3.3:** Details of experimental design for evaluating captive breeding performances

| ***Treatment*** | ***Replications*** | ***Description*** |
|---|---|---|
| Aquarium (1 female and 2 males) | | |
| $T_1$ | 3 | Observation of natural breeding in aquaria, no hormone was administered |
| $T_2$ | 3 | Study of breeding performances after injecting PG hormones at different doses (5 mg/kg for female and 2 mg/kg for male) into glass aquaria |
| $T_3$ | 3 | Study of breeding performances after injecting synthetic hormones (*Ovulin*) at 0.5 ml/kg into glass aquaria |
| Earthen ponds (3 pairs) | | |
| $T_1$ | 3 | Observation of natural breeding in experimental ponds, no hormone was administered |
| $T_2$ | 3 | Study of breeding performances after injecting PG hormones at different doses (5 mg/kg for female and 2 mg/kg for male) into earthen experimental ponds |
| $T_3$ | 3 | Study of breeding performances after injecting synthetic hormones (*Ovulin*) at at 0.5 ml/kg into earthen experimental ponds |

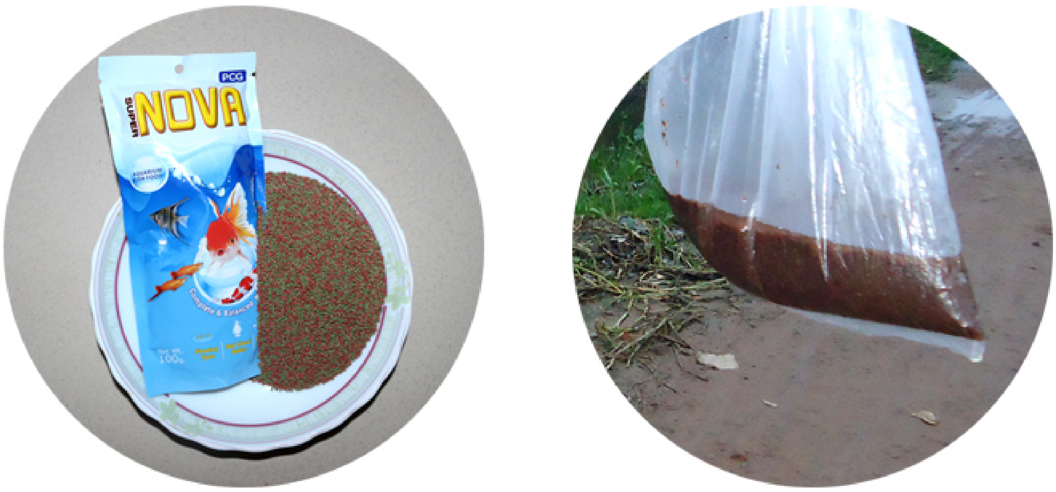
## 3.9 Observation of breeding and larval development

Courtship and other breeding behaviors were observed in experimental aquaria. Continuous monitoring of breeding tanks was performed during the breeding time.

Unfortunately, there was no suitable device or equipment at the laboratory of the Department of Fisheries and thus it was not possible to take digital images of the larval development of the studied species. The larval development stages were observed under the light microscope and hand-drawing images were produced to describe the overall larval developmental phages. The drawings were then scanned using a scanner (model HP Deskjet F2182 All in One Printer Scanner Copier) and further edited by computer software Adobe Photoshop (version CS). Measurement of the total length of specimens was taken after being immobilized in cooled water which made the accurate observation of specimens possible without causing any shrinkage due to death, anesthetics and preservatives.

## 3.10 Feeding

In earthen experimental ponds breeding trials were primarily dependent on natural foods. To enhance the growth of natural food, *i.e.* plankton, regular fertilization was practiced. In organic fertilizer TSP (triple super phosphate, at the rate of 24.7 kg/ha) and urea (at the rate of 12 kg/ha) were broadcast on weekly basis. However, on the other hand in aquaria daily feeding with commercial aquarium fish feeds was practiced twice a day (in morning at 10:00 and at night at 20:00 hour). Commercial feeds were supplied in both crushed and original forms in order to ensure maximum and easy consumption for the newly born larvae. In addition to this, boiled egg yolk and *Tubifex* sp. were also supplied once a day. The boiled egg yolk was first diluted in potable water before supply.




(a) Commercial aquarium feed (b) *Tubifex* in polythene bag

**Figure 3.13:** Different feeds supplied to the young, brood and larvae

## 3.11 Survival status

Survival rate of the newly born larvae was determined in order to understand the breeding success in captivity. Survival rate was determined for the larvae hatched in the experimental aquaria only. Due to limited laboratory facilities it was not possible to calculate the survival status of fishes in experimental ponds.

The survival rate of banded/giant and dwarf gouramies was calculated using the following formula-

Survival rate (%) = $\frac{Number\ of\ live\ individuals\ of\ fish\ species}{Number\ of\ Initial\ fish\ individuals}$x100 (Mannan *et al.* 2012)

### 3.12 Water quality analysis

Important water quality parameters were measured using standard techniques. The studied water quality parameters included dissolved oxygen (DO), free carbon dioxide ($CO_2$), alkalinity, ammonia, pH, water depth of pond, water transparency of experimental ponds and water temperature.

The DO, free $CO_2$, alkalinity, ammonia and pH were measured according to the guideline of Hach (1991). A HACH Kit Box (Model FF-2; USA) was used for the evaluation of these water quality parameters. Water depth of experimental ponds was determined using a measuring stick. A digital thermometer was used to determine the water temperatures of both pond and aquaria. By means of a Secchi disc water transparency was measured to the nearest mm in experimental ponds.

### 3.13 Benefit-cost ratio (BCR) analysis

In the last two years of the research *i.e.* 2014 and 2015; young produced under this research project were supplied to the local aquarium shops of Rajshahi city. Benefit-cost ratio of different treatments was also determined to find out the most profitable treatment in terms of money. However, the following formula was used to calculate BCR,

$BCR = \frac{B}{K}$ (FAO, 1991)

Here, B = net benefit and K = sum of cost

## 3.14 Statistical analysis

Statistical analyses were done using Statistical Package for Social Science (SPSS) version 15.0 for Windows and Microsoft Excel 2007/2010. Collected data were subjected to One Way Analysis of Variance (ANOVA; Tukey HSD), Paired Sample t-test and other standard analyses to find out statistically significant differences among various treatments. However, simple descriptive analyses were also conducted wherever needed.

## 3.15 Materials used

Various materials were used to carry out the research program. A short list of such materials is presented here in Table 3.4.

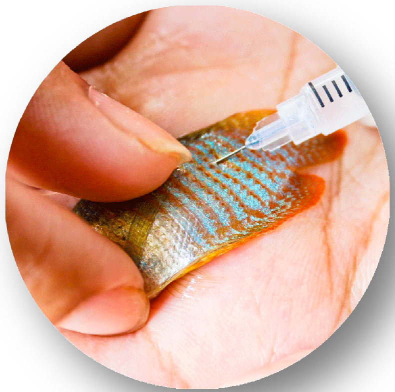
**Table 3.4:** List of materials used in the present study

| *Serial* | *Name* | *Purpose* |
|---|---|---|
| 01. | Aquarium | To carry out breeding programs and for brood and young rearing |
| 02. | Hormones | For induced breeding of the fish species |
| 03. | Syringe | For hormone injection |
| 04. | Digital balance | To determine the weight of fish and ovary |
| 05. | Measuring scale | To measure the length of fishes |
| 06. | Light and simple microscope | To observe the larval development |
| 07. | HACH kit box, thermometer and secchi disc | To determine status of water quality parameters |
| 08. | Digital camera | For the purpose of capturing photographs |
| 09. | Note book, pencil, pen, eraser and other related materials | For documentations |

Captive breeding of *Trichogaster lalius* (Hamilton, 1822) and *Trichogaster fasciata* Bloch and Schneider, 1801

## CHAPTER FOUR

# RESULTS AND DISCUSSION



# RESULTS AND DISCUSSION

## 4.1 Length and weight of fishes prior to breeding trial

After collecting from the wild sources, the collected specimens were reared for about a year in laboratory conditions. Prior to breeding trial in captivity the mean length of male banded gourami, *Trichogaster fasciata* was found 7.94±2.36 cm, whereas, the mean weight was 10.10±1.45 g. The mean length and weight of female *T. fasciata* were recorded as 8.23±2.31 cm and 11.98±1.12 g (Figure 4.1 and 4.2).

In case of male dwarf gourami, *T. lalius* the total length and body weight were found 5.94±1.99 cm and 7.14±1.33 g respectively. On the other hand, mean total length and weight of female dwarf gourami were found as 6.02±2.13 cm and 7.84±1.56 g respectively (Figure 4.1 and 4.2).

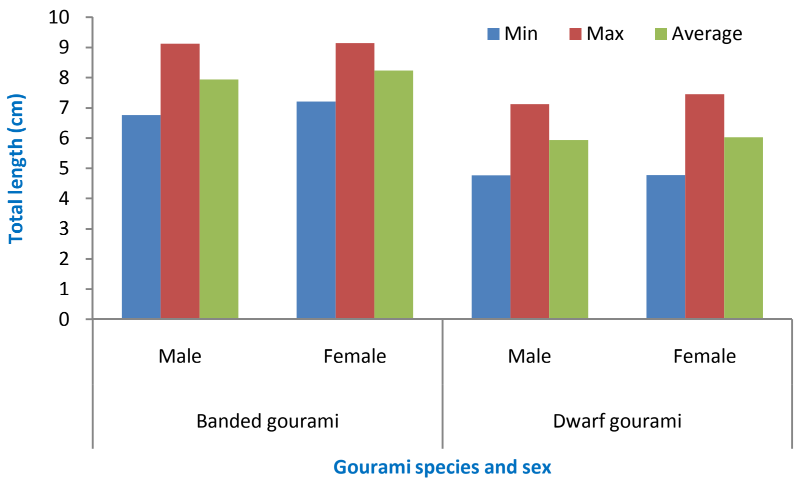



**Figure 4.1:** Total length of the two gourami species prior to breeding trial after about one year of rearing in the laboratory

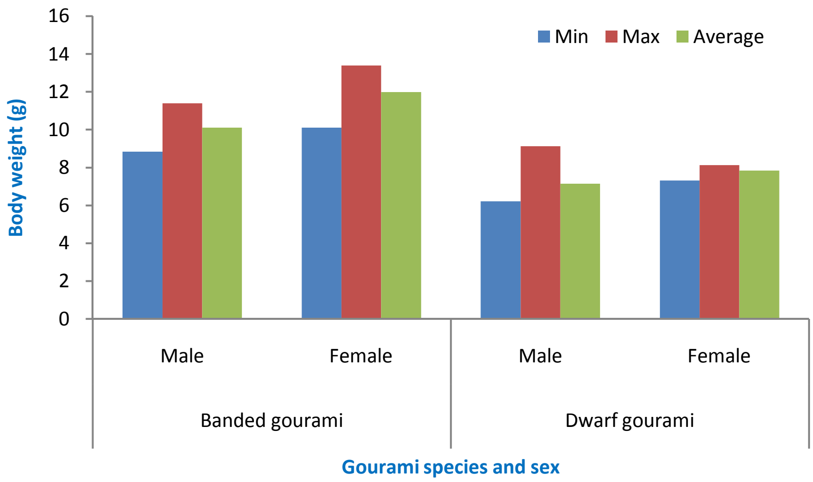



**Figure 4.2:** Body weight of the two gourami species prior to breeding trial after about one year of rearing in the laboratory

In a study by Hossen *et al.* (2014), the total length and weight of female was found as 8.7±1.2 cm and 17.41±1.3 g respectively. Whereas, total length and body weight of male were 10.0±1.3 cm and 19.25±1.51 g respectively. These results do not comply with the findings found in the present research. Though total length of fish is found more or less similar but weight detected in research by Hossen *et al.* (2014) was much more than that of present findings. However, it is quite unusual, in study by Hossen *et al.* (2014), that a mature male individual of smaller total length gained more body weight than that of a ripe female of larger size (total length) during breeding season.

Rahman (1989 and 2005) mentioned that *T. fasciata* and *T. lalius* can attain total length of over 10 cm and 8.8 cm respectively. Talwar and Jhingran (1991) mentioned that *T. fasciata* can grow up to 12 cm in total length. Both are little larger in size compared to the size grown in the present experiment. However, Bhuiyan (2964) collected *T. fasciata* of maximum 8 cm in total length which was smaller than that of fishes grown in the present research.

In a research conducted by Galib (2008) the highest total lengths measured for *T. lalius* and *T. fasciata* were 5 cm and 6 cm respectively in the Chalan Beel of northwest part of Bangladesh which are quite similar to the present findings. Another research by Mitra *et al.* (2007) revealed that female of *T. fasciata* mature at 5.7 cm in total length indicating all the specimens used in the present research were sexually matured.

## 4.2 Fecundity

Very strong correlation was found between body weight and fecundity of the studied species which are depicted in Figure 4.3 and 4.4. Mean individual fecundity was found 1273±516.81 in giant gourami (*T. fasciata*) with the minimum and maximum fecundity of 2163 and 565 respectively. On the other hand, in case of dwarf gourami (*T. lalius*), mean fecundity was calculated 160.50±26.42 with the highest 209 and the lowest 115 eggs per individual.

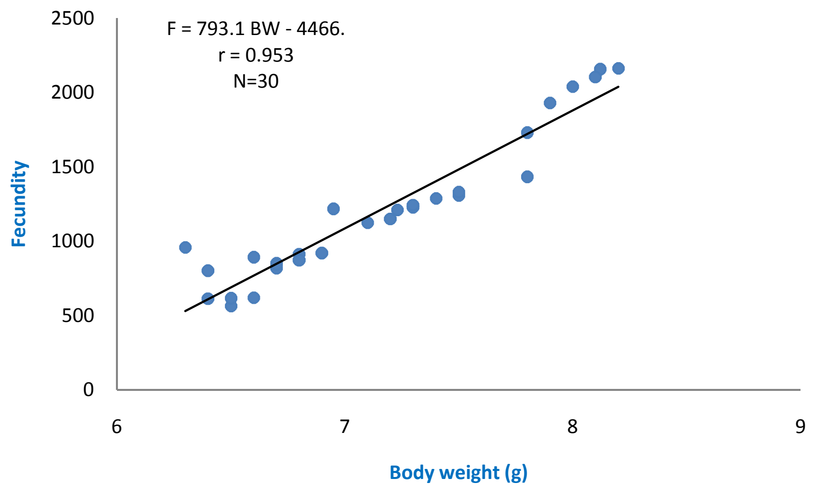



**Figure: 4.3:** Relationship between fecundity and body weight of banded or giant gourami, *Trichogaster fasciata*

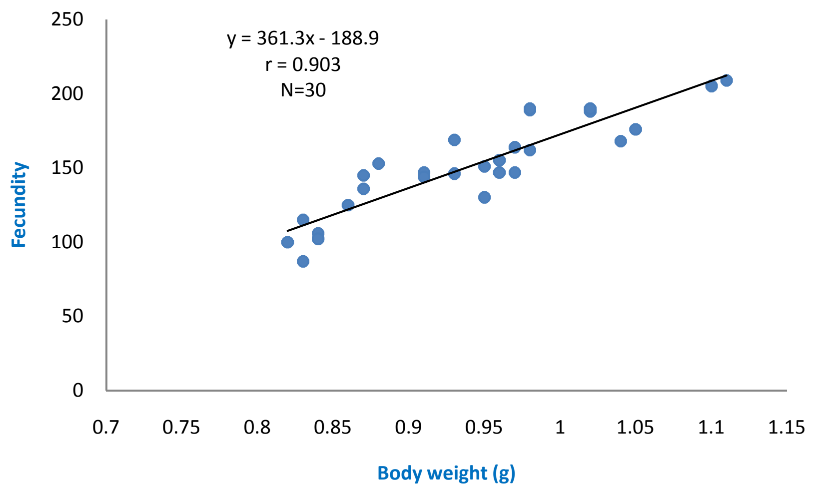



**Figure: 4.4:** Relationship between fecundity and body weight of dwarf gourami, *Trichogaster lalius*

The sexual maturity and fecundity of an individual of an individual female varies with many factors- for example size, age, type of species and surrounding condition like availability of food and environmental parameters (Lagler *et al.* 1977). Saha (2007) mentioned that the fecundity of *T. fasciata* varies from 500 to 1000 which was not similar to the findings of the present research. In this research more fecundity was recorded. Saha (2007) also mentioned that female *T. lalius* can produce about 600 eggs which was much more higher than that of present findings. Behra *et al.* (2005) recorded a fecundity range of 599-5522 (5.9-7.2 cm in total length and 7.3-10.8 g in weight). This fecundity range was not similar to the fecundity recorded in the present study.

In another investigation by Mitra *et al.* (2007), the fecundity of *T. fasciata* was recorded 1095 to 19291 eggs which were far more than that of the fecundity recorded in the present study. They have also stated that fecundity of this species was high during April to September. However, all these efforts suggest that the fecundity of gouramies vary greatly. According to Wotton (1973) and Hoque and Rahman (2008), variation in fecundity is primarily a reflection of variation in the size of fish at sexual maturity.

## 4.3 Breeding performances

Breeding performances of the gouramies under different treatments in both aquaria and experimental ponds are presented below.

### 4.3.1 Breeding in aquaria

In case of giant gourami, *T. fasciata*, the highest number of individuals (345±49.93) was obtained in the $T_3$ in 2014. Whereas, the lowest number of individuals (00±00.00) were found in $T_2$ in 2013. Breeding pairs did not spawn in $T_2$ in that year (2013). ANOVA analyses have revealed that all the results found in case of $T_1$ and $T_3$ were significantly different from $T_2$ ($p$<0.05) (Table 4.1).

On the other hand, in case of dwarf gourami, *T. lalius*, the highest number of individuals (79.67±11.85) was obtained in the $T_3$ in the first year of captive breeding study *i.e.* 2013. Whereas, the lowest number of individuals (00±00.00) were found in $T_2$ in 2013. Just like $T_2$ for giant gourami, breeding pairs did not spawn in $T_2$ in 2013 too. ANOVA analyses have revealed that all the results found in case of $T_1$ and $T_3$ were significantly different from $T_2$ ($p$<0.05) (Table 4.1).

**Table 4.1:** Breeding performances of gouramies in aquaria

| Fish species | Year | Number [Mean±SD(SE)] | | |
|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_3$ |
| *T. fasciata* | 2013 | 268±124.81 (72.06)$^{b}$ | 00±00.00 (0.00)$^{a,c}$ | 307±75.62 (43.66)$^{b}$ |
| | 2014 | 322±38.97 (22.50)$^{b}$ | 17±17(9.81)$^{a,c}$ | 345±49.93 (28.83)$^{b}$ |
| *T. lalius* | 2013 | 53.67±11.68 (6.74)$^{b,c}$ | 00±00.00 (0.00)$^{a,c}$ | 79.67±11.85 (6.84)$^{a,b}$ |
| | 2014 | 52.67±7.09 (4.10)$^{b}$ | 8.67±8.50 (4.91)$^{a,c}$ | 69.33±7.77 (4.48)$^{b}$ |

***p* = 0.05**
**a**, results significantly varies with $T_1$;
**b**, result significantly varies with $T_2$;
**c**, result significantly varies with $T_3$

### 4.3.2 Breeding in experimental ponds

In case of giant gourami, *T. fasciata*, the highest number of individuals (1532±249.31) was obtained in the $T_1$ in 2014. Whereas, the lowest number of individuals (00±00.00) were found in $T_2$ in 2013. Breeding pairs did not spawn in $T_2$ in that year (2013). ANOVA analyses have revealed that all the results found in case of $T_1$ and $T_3$ were significantly different from $T_2$ ($p<0.05$) (Table 4.2).

On the other hand, in case of dwarf gourami, *T. lalius*, the highest number of individuals (265±39.15) was obtained in the $T_1$ in the second year of captive breeding trial *i.e.* 2014. Whereas, the lowest number of individuals (00±00.00) were found in $T_2$ in 2013. Just like $T_2$ for giant gourami, breeding pairs did not spawn in $T_2$ in 2013 too. ANOVA analyses have revealed that all the results found in case of $T_1$ and $T_3$ were significantly different from $T_2$ ($p<0.05$) (Table 4.2).

**Table 4.2:** Breeding performances of gouramies in ponds

| Fish species | Year | Number [Mean±SD(SE)] | | |
|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_3$ |
| *T. fasciata* | 2013 | 1487±256.08 (147.85)$^{b}$ | 00±00.00 (0.00)$^{a,c}$ | 1157±73.51 (42.44)$^{b}$ |
| | 2014 | 1532±249.31 (143.94)$^{b}$ | 266±39.04 (22.54)$^{a,c}$ | 1087±110.49 (63.79)$^{b}$ |
| *T. lalius* | 2013 | 249±53.08 (30.64)$^{b}$ | 00±00.00 (0.00)$^{a,c}$ | 188±52.16 (30.12)$^{b}$ |
| | 2014 | 265±39.15 (22.61)$^{b}$ | 90±24.56 (14.18)$^{a,c}$ | 220±23.30 (13.45)$^{b}$ |

***p* = 0.05**
**a**, results significantly varies with $T_1$;
**b**, result significantly varies with $T_2$;
**c**, result significantly varies with $T_3$

No previous studies on captive breeding of *T. lalius* and *T. fasciata* were recorded in Bangladesh. So, this was not possible for the researcher to compare the present findings with the previous one. However, one research on seed production of these two species has been carried out in India by Das and Kalita (2006) but no specific production performances were mentioned in their report. However, they mentioned that 100-150 seeds can be raised in an aquarium of 80x40x40 cm size which is much lower than that of present findings for *T. fasciata* in two treatments ($T_1$ and $T_3$) but higher than $T_2$. No previous statistics were found for breeding performance in earthen pond and thus, the findings of the present study could not be compared against a previous one. Breeding in aquaria set to direct exposure of sunlight would give maximum breeding performance as this helps to create an environmental inside the aquarium similar to that of wild.

In the present study, floating aquatic vegetations (*Pistia* sp. and others) were provided in aquaria to create suitable environment for natural breeding of gouramies. Similar comment was also made by Das and Kalita (2006) who stated that thick aquatic weeds need to be provided with sufficient space for movement of larvae. However, *T. fasciata* can survive and breed in such water bodies where many other species may not survive (Bhuiyan 1964).

Hossain (2014) reported that breeding protocol has been developed for *T. fasciata* in Bangladesh but no other information was published by him in this regard. He also mentioned that about 20 fish species have been domesticated and their breeding and rearing protocols have been developed, of which, 50% are Cypriniformes and now under nation-wide aquaculture. However, *T. fasciata* was not brought under aquaculture till date and mass production of seeds was absent in Bangladesh.

In India, Abujam *et al.* (2015) carried out research on the captive breeding of *T. fasciata* using inducing hormone (ovaprim). In their research, success of breeding was reported in all the breeding trials but in the present research poor or sometimes no results were found for PG injection. There might be something wrong with the PGs used in the present research due to which the studied species showed poor or sometimes no response to this hormone.

## 4.4 Larval development

Early larval development states of both dwarf gourami and banded gourami were observed in the present research. The findings of this investigation are described here under different headings.

### 4.4.1 Early larval development of dwarf gourami, *Trichogaster lalius*

Table 4.3 shows the different distinguishing characteristics of *T. lalius* larvae. Hand-drawing of five different early developmental stages are shown in Figure 4.5. Immediately after hatching, the larvae were with large yolk sac and melanophores were more in the yolk sac region (Figure 4.5, a-b). At the stage of hatching, the total length of dwarf gouramies varied between 3.0 and 3.25 mm, the body of the larvae remained slender with a very long tail (Figure 4.5, a-e). The anus was found well forward around the middle portion of the total length. Pectoral fins in some specimens were well developed, although other fins remained indistinguishable from the primordial fin. Pigmentation became prominent in the head region in all the specimens examined. Larvae, aged between 10 and 13 days, were with very slender body and conspicuous eye spots (Figure 4.5, e).

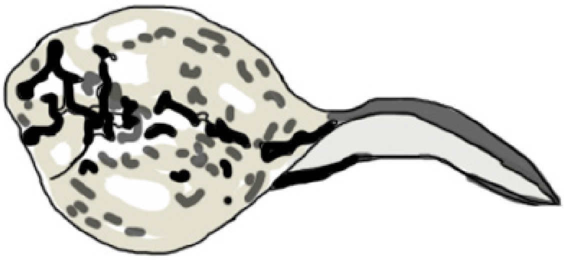
The larvae of over 20-day old showed clear morphological features. Melanophores and fin differentiation were found so prominent in all the specimens studied. A post larva of around 4.5 mm long showed a similar pigmentation pattern to the newly hatched post larva except for an increase in number of dorsal and ventral contour and mediolateral melanophores. Around 6.0 mm long post larva with evident coloration showed the presence of rays in the dorsal, anal and caudal fins. A row of internal notochord melanophores was also seen at this stage.

**Table 4.3:** The distinguishing characteristics of dwarf gourami, *Trichogaster lalius* larvae

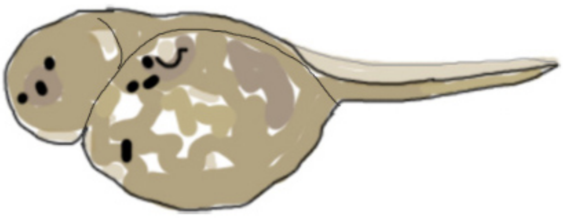
| ***Time after hatching*** | ***Characters*** |
|---|---|
| 1-2 hours | Large yolk sac, a long elongated tail. More pigmentation at yolk sac portion. Larvae almost inactive. |
| 6-7 hours | Large yolk sac, a long elongated tail. More pigmentation in anterior portion of the body, *i.e.* head and yolk sac. Larvae almost inactive. |
| 40-60 hours | Comparatively smaller yolk sac, almost uniform pigmentation all over the body. |
| 72-96 hours | Small yolk sac and conspicuous eye spots. Much active larvae. |
| 120-150 hours | Larvae with well or full pigmented eyes and no yolk sac. Sign of notochord. |
| 240-320 hours | Larvae with large eye spots and slender elongated body. Notochord clearly conspicuous. |
| 480-550 hours | Almost laterally compressed larvae with elongated body. Caudal fin with conspicuous fin rays. Symbol of the development of other fins and rays. |

No previous study on larval development of dwarf gourami, *T. lalius* was found, thus, it was not possible for the researcher to compare the present findings with the previous one.

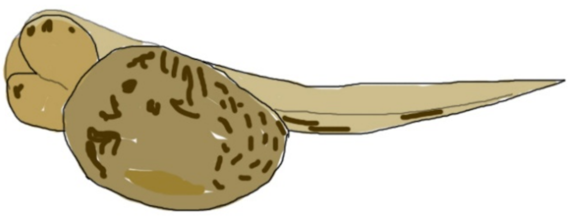
***(a)*** Hatchling (1 to 2 hours)



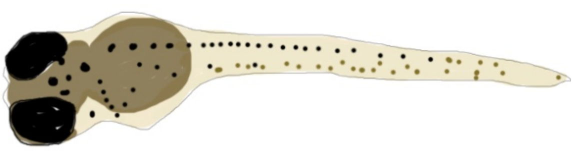
***(b)*** Advanced hatchling (6 to 7 hours)



***(c)*** Larva with yolk sac



***(d)*** Larva of 3rd to 4th days



***(e)*** 10 to 13-day old larva

**Figure 4.5:** Different stages of early larval development of dwarf gourami, *Trichogaster lalius*

### 4.4.2 Early larval development of giant/banded gourami, *Trichogaster fasciata*

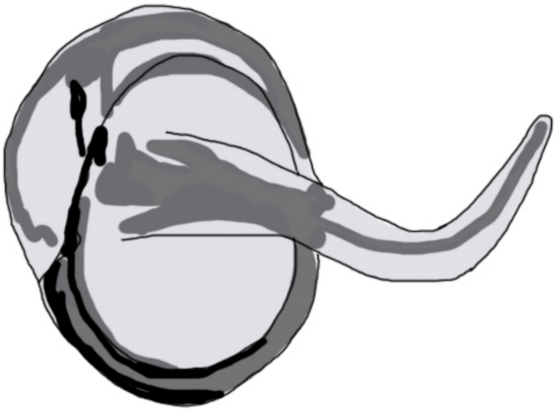
Table 4.4 shows the different distinguishing characteristics of *T. fasciata* larvae. Hand-drawing of five different early developmental stages are shown in Figure 4.6. Immediately after hatching, the larvae were with large yolk sac and melanophores were more in the yolk sac region (Figure 4.6, a-b). At the stage of hatching, the total length of banded gouramies varied between 3.5 and 4.5 mm, the body of the larvae remained slender with a very long tail (Figure 4.6, a-e). The larva was generally with sign of un-pigmented eye spot. In the early state of larval development no sign of fins were observed. Later, pectoral fins in some specimens were found well developed, although other fins remained indistinguishable from the primordial fin. Pigmentation became prominent in the head region in all the specimens examined. After 30 hours, the larvae reached over 4 mm in total length.

Larvae, aged between 10 and 13 days, were with very slender body and conspicuous eye spots (Figure 4.6, e). The larvae of over 20-day old showed clear morphological features. Melanophores and fin differentiation were found so prominent in all the specimens studied. A post larva of around 5.5 mm long showed a similar pigmentation pattern to the newly hatched post larva except for an increase in number of dorsal and ventral contour and mediolateral melanophores. A row of internal notochord melanophores was also seen at this stage.

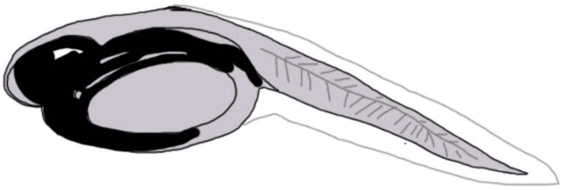
**Table 4.4:** The distinguishing characteristics of dwarf gourami, *Trichogaster fasciata* larvae

| *Time after hatching* | *Characters* |
|---|---|
| 1-2 hours | Large yolk sac, a long elongated tail. More pigmentation at yolk sac portion. Larvae almost inactive. No conspicuous eye spot. |
| 6-7 hours | Large yolk sac, a long elongated tail. More pigmentation in anterior portion of the body, *i.e.* head and yolk sac. Sign of mouth formation. |
| 15-16 hours | Large yolk sac, more pigmentation. Sign of heart and gut. |
| 24-26 hours | Smaller yolk sac. Early sign of notochord. More pigmentation. Prominent eye spots. |
| 36-40 hours | Smaller Yolk sac. Conspicuous eye. Sign of pectoral fins. Sign of gill opening. |
| 28-56 hours | Very small yolk sac. Fully active larvae. Well developed mouth. More pigmentation. |

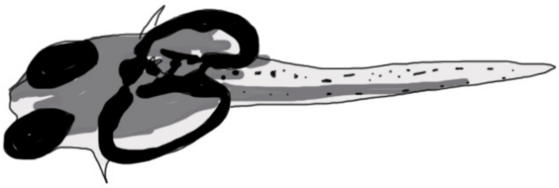
***(a)*** Hatchling



***(b)*** 15 to 16-hour old larva



***(c)*** 24 to 26-hour old larva



***(d)*** 3 to 4-day old larva

***(e)*** 20 to 23-day old larva

**Figure 4.6:** Different stages of early larval development of banded gourami, *Trichogaster fasciata*

Studies to reveal the larval development of gouramies are seldom found. The present research is the first study in which larval development of both banded/giant gourami (*T. fasciata*) and dwarf gourami (*T. lalius*) were studied and described.

In a research by Hossen *et al.* (2014) embryonic and larval development of *T. fasciata* were studied but their study did not include *T. lalius*. In a research by Motilan and Nishikanta (2014) described the larval development of another gourami species *T. labiosua* in India.

## 4.5 Survival status

During the preliminary breeding trial to determine the standard doses of the hormones for the studied gourami species it was not possible to keep the newly born larvae alive for more than 13 days. But when the experimental aquaria were set at such a place where direct sunlight was available, the survival of both species of gouramies was found remarkably higher (Figure 4.7 and 4.8).

### 4.5.1 Survival of banded/giant gourami larvae

In case of the banded gourami, maximum 50% survival rate was observed in $T_3$ on the $10^{th}$ day after hatching followed by $T_1$ (47% survival). Unfortunately very little survival rate was found in $T_2$ where about 3.5% survival was recorded on the $10^{th}$ day after hatching of larvae which further reduced to below 1% on the $30^{th}$ day. After one month of hatching, almost similar survival rate of newly born larvae was observed in both $T_1$ and $T_3$ (Figure 4.7). Paired sample t-test results have shown survival rate in $T_2$ was significantly different from other two treatments, *i.e.* $T_1$ and $T_2$ ($p<0.05$).

### 4.5.2 Survival of dwarf gourami larvae

In comparison to the banded gourami higher survival was observed in case of dwarf gourami. In case of the dwarf gourami, about one-third of the total larvae survived till the $10^{th}$ day after hatching in $T_3$ followed by $T_1$ where about 60% larvae survived. Very little survival rate was found in $T_2$ just like the banded gourami. After one month of hatching, over half of the larvae survived in $T_3$ and over one-third of the larvae survived in $T_1$ (Figure 4.8). However, paired sample t-test results have shown survival rate in $T_2$ was significantly different from other two treatments, *i.e.* $T_1$ and $T_2$ ($p<0.05$), similar to that of survival of *T. fasciata*.

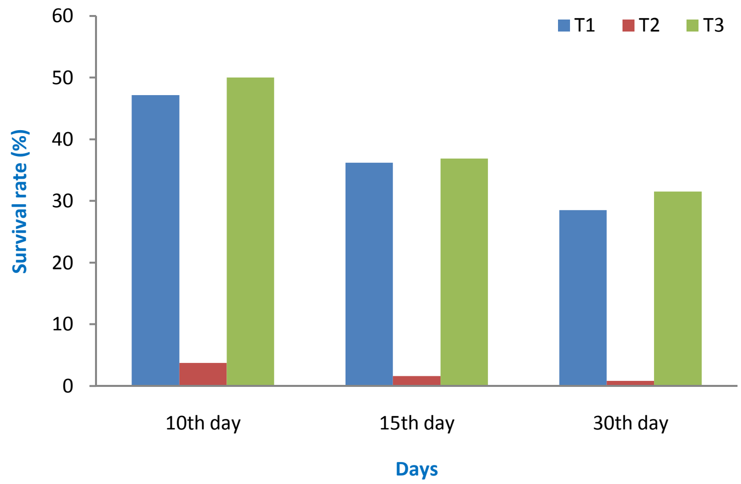



**Figure 4.7:** Survival of banded gourami, *Trichogaster fasciata* larvae in aquaria

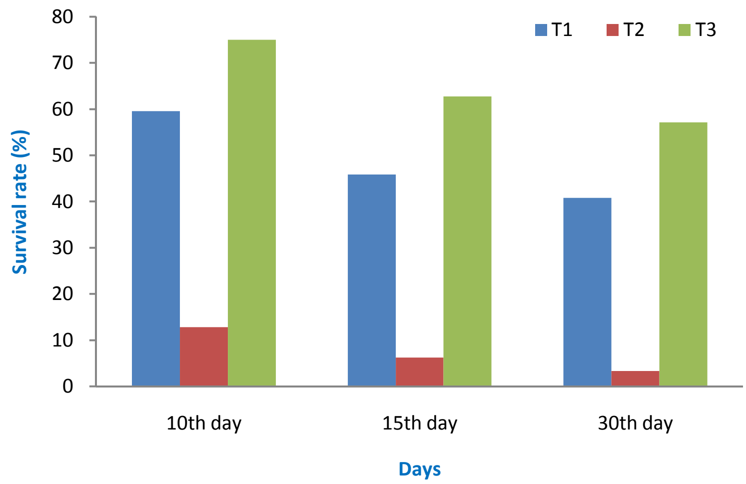



**Figure 4.8:** Survival of dwarf gourami, *Trichogaster lalius* larvae in aquaria

No previous studies were found by the research regarding survival of banded and dwarf gouramies produced in captivity. Thus, it was not possible to compare the present results with previous findings. Afroz *et al.* (2014) conducted a study on *Mastacembelus pancalus* and produced larvae in captivity in Bangladesh but the larvae did not survive for more than 17 days in experimental aquaria. However, no such mortality was recorded in the present study.

## 4.6 Water quality parameters

Breeding of any fish is regulated by several environmental factors, such as photoperiod, temperature, rainfall and food. Various water quality parameters were measured during the breeding period of gouramies. The recorded results are presented here under different subheadings.

### 4.6.1 Water temperature

Table 4.5 shows the mean value of water temperature in all the treatments, in both aquaria and experimental earthen ponds. Water temperature level of aquaria was found slightly lower than that of ponds. The highest temperature in aquaria was 28.50±1.07 (in $T_3$, 2014) and the lowest was 27.61±1.63 (in $T_3$, 2013). In case of ponds, the highest and lowest mean temperature was recorded 31.79±1.42 (in $T_3$, 2014) and 31.36±1.56 (in $T_2$, 2014). However, no statistically significant differences were found among treatments of aquaria and earthen ponds (*p*>0.05) (Table 4.5).

**Table 4.5:** Mean (±SD) water temperature (°C) in the breeding environments

| ***Year*** | ***Aquaria*** | | | ***Experimental ponds*** | | |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_1$ | $T_2$ | $T_3$ |
| 2013 | 27.93±1.44 | 27.82±1.27 | 27.61±1.63 | 31.54±1.52 | 31.36±1.56 | 31.50±1.61 |
| 2014 | 27.82±1.27 | 27.86±1.12 | 28.50±1.07 | 31.39±1.47 | 31.43±1.25 | 31.79±1.42 |

*No statistically significant differences among treatments in both aquaria and earthen ponds in both years (p>0.05)*

Water temperature of the breeding environments, both aquaria and ponds, are shown in Figure 4.9 and 4.10. In case of breeding aquaria, the highest water temperature was recorded 31 °C in $T_1$ in 2013. Whereas, the lowest water temperature was found 24 °C in $T_3$ in 2013. The mean temperature was 27.92 °C (Figure 4.9 and 4.10).

On the other hand, in ponds, the mean water temperature was found 31.50 °C which was higher than that of aquaria. The highest and lowest temperatures were recorded 34.50 °C (in $T_3$ in 2013) and 28.50 (in $T_3$ in 2013) respectively (Figure 4.9 and 4.10).

According to Axelrod and Shaw (1967), fish species can breed at temperatures between 18 and 29°C. In a study by Das and Kalita (2006), water temperature of the breeding tanks from 25-27 °C gave expected output and they have recommended it as suitable for breeding of gouramies. However, this range of water temperature was slightly lower than that of water temperature recorded in the present study. Geographical location may play an important role in this case, as the study of Das and Kalita (2006) was conducted in Assam of India and the present research was carried out in Rajshahi of Bangladesh.

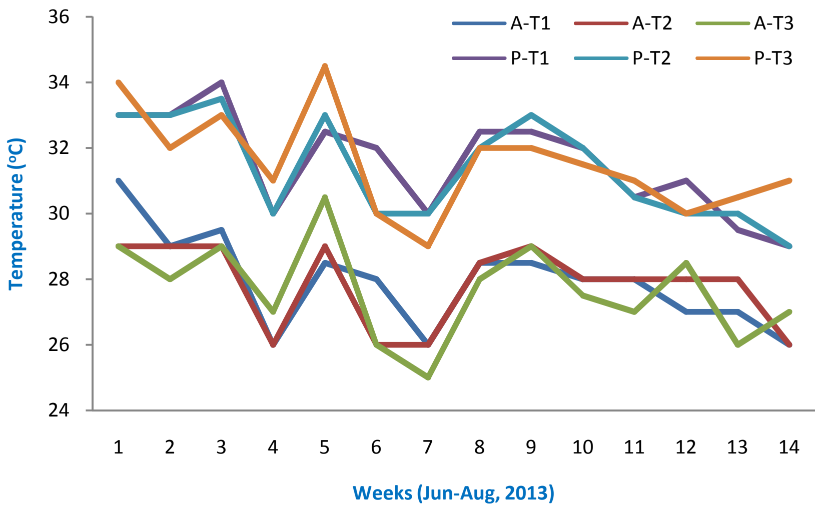



**Figure 4.9:** Water temperature in breeding aquaria and ponds in 2013; A=Aquaria, P=Pond and T=Treatment

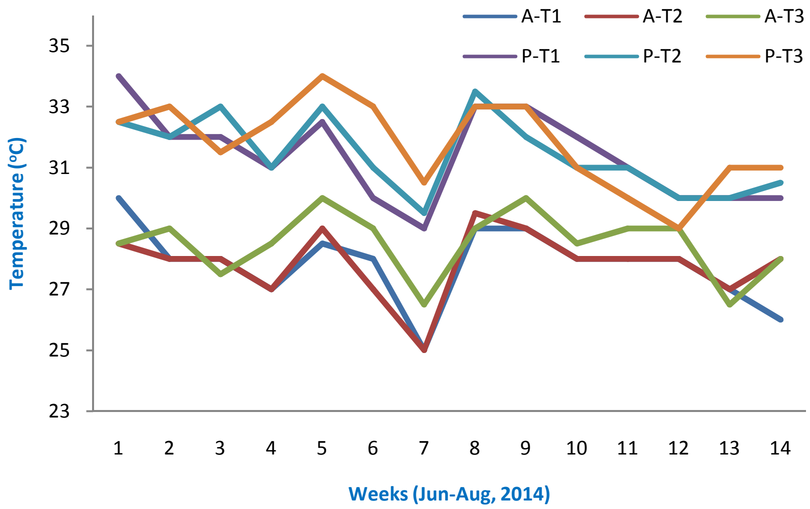



**Figure 4.10:** Water temperature in breeding aquaria and ponds in 2014; A=Aquaria, P=Pond and T=Treatment

Abujam *et al.* (2015) conducted a study on breeding of banded gourami, *T. fasciata* using ovaprim hormone in India and found that water temperature during the breeding trial varied from 20 °C to 29 °C, from December to May. In comparison to this observation, water temperature was found higher in the present study especially in experimental ponds. Temperature recorded in the present study is similar to the natural aquatic habitats of Bangladesh (e.g. Chakraborty *et al.* 2012b).

Afroz *et al.* (2014) stated that water temperature between 27 °C and 30 °C is suitable for breeding of spiny eel, *Mastacembelus pancalus*. Research efforts revealed that water temperature of 25-27 °C is suitable for *Macrognathus aculeatus* and 27-31 °C for *M. pancalus* (Rahman *et al.* 2009). For breeding of gourami the temperature should be above 26°C (79°F), temperature preferably around 28-30 °C during spawning (Axelrod, 1980). So, water temperature recorded in the present study was suitable for the breeding of gouramies. In another research, Mohsin and Mondal (2013) reported that water temperature in aquarium varied from 25.66±0.17 to 28.66±0.35°C during their work on survival of exotic ornamental fishes of Bangladesh which is quite similar to the present findings.

### 4.6.2 Water transparency

Water transparency of the breeding ponds was recorded only in experimental earthen ponds, which are shown in Figure 4.11 and 4.12. The mean water transparency was found 33.56 cm with the highest transparency of 45 cm in $T_2$ in 2013 and the lowest 29 cm in $T_3$ in 2014 (Figure 4.11 and 4.12).

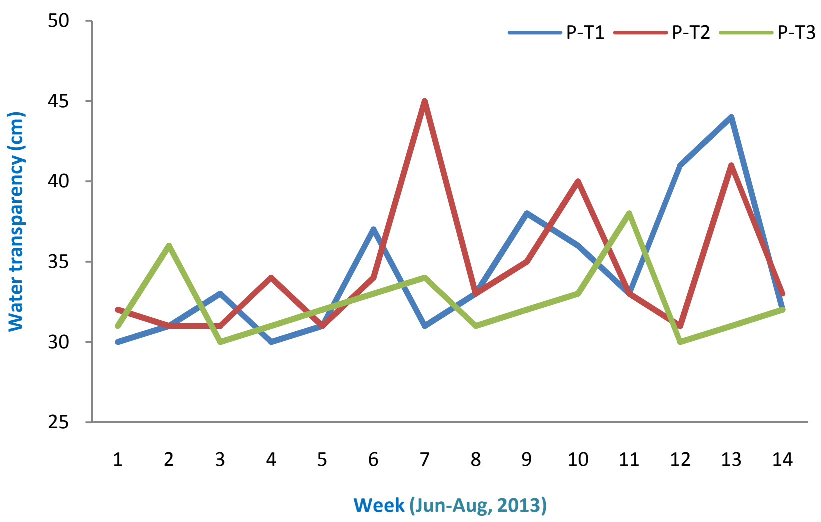



**Figure 4.11:** Water transparency in breeding ponds in 2013; A=Aquaria, P=Pond and T=Treatment

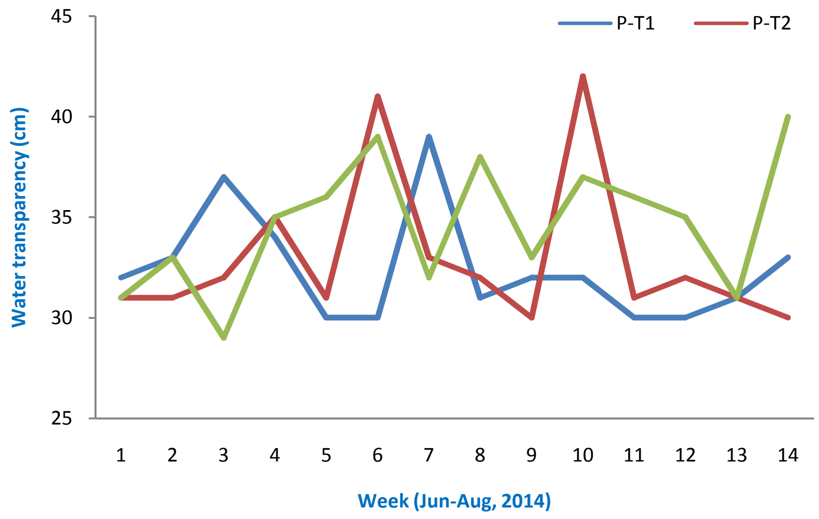



**Figure 4.12:** Water transparency in breeding ponds in 2014; A=Aquaria, P=Pond and T=Treatment

Water transparency was measured only in experimental earthen ponds (Table 4.6). No major variation was observed in the value of water transparency in two years of study. The highest mean transparency was found 34.64±3.27 cm in $T_3$ (2014) whereas, the lowest value (32.43 cm) was recorded in $T_3$ (2013) and $T_1$ (2014) (Table 4.6). However, no statistically significant differences were found among treatments of aquaria and earthen ponds ($p$>0.05)

**Table 4.6:** Mean (±SD) water transparency in different treatments of experimental ponds

| ***Year*** | ***Water transparency (cm) in experimental ponds*** | | |
|---|---|---|---|
| | ***$T_1$*** | ***$T_2$*** | ***$T_3$*** |
| 2013 | 34.29±4.32 | 34.57±4.34 | 32.43±2.28 |
| 2014 | 32.43±2.71 | 33.00±3.82 | 34.64±3.27 |

*No statistically significant differences among treatments (p>0.05)*

The water transparency level recorded in the present research is little higher than that of the findings of Chakraborty *et al.* (2012b) who recorded 20.22 cm to 31.86 cm water transparency in a wetland of Bangladesh. Siddique *et al.* (2010) reported the lowest and highest water transparency of 26.72 cm and 40.13 cm respectively in a fish pond which is similar to the present findings. Water transparency of 40 cm or less is considered standard for aquatic habitats (Rahman 1992) so the water transparency level recorded in the present study was within the suitable range. However, as per FAO (1997), 40 to 60 cm water transparency is considered best for fish production but 15 to 40 cm water transparency was recommended suitable by Boyd (1982).

### 4.6.3 Water depth

Like water transparency, water depth of the breeding ponds was recorded only in experimental earthen ponds, which are shown in Figure 4.13 and 4.14. The mean water depth in earthen ponds was found 4.39 feet with the highest depth of 5.70 feet in $T_2$ in 2013 and the lowest 3.20 feet in $T_1$ in 2013 (Figure 4.13 and 4.14).

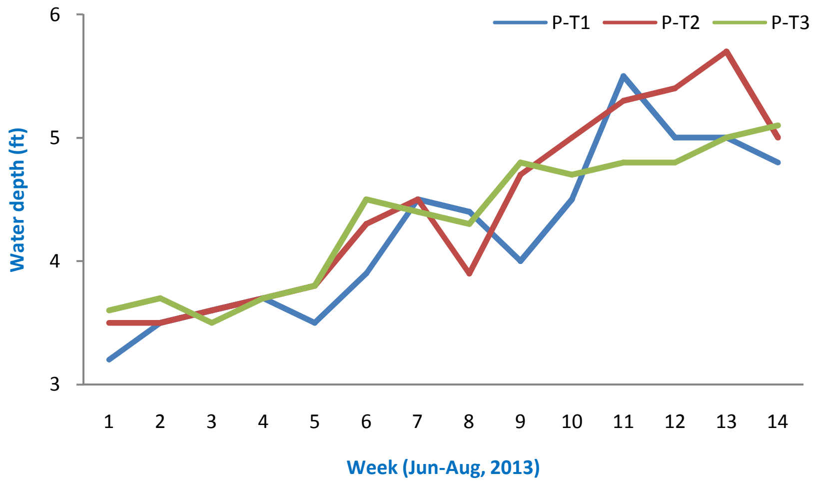



**Figure 4.13:** Water depth in breeding ponds in 2013; A=Aquaria, P=Pond and T=Treatment

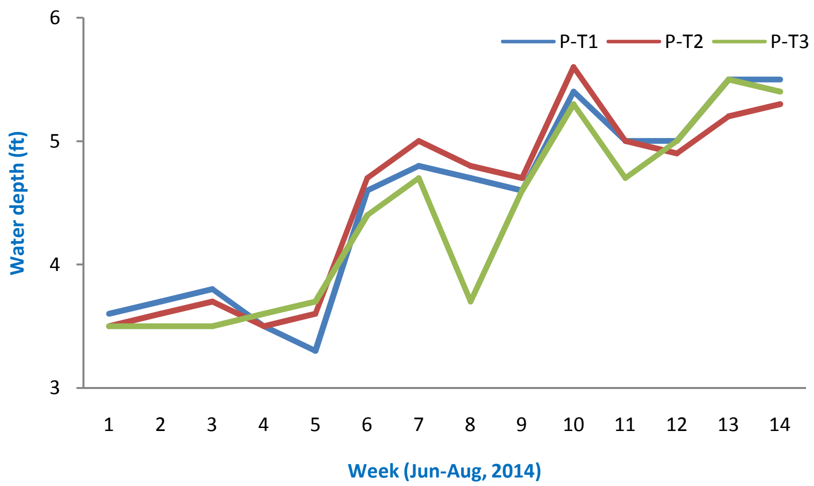



**Figure 4.14:** Water depth in breeding ponds in 2014; A=Aquaria, P=Pond and T=Treatment

Water depth was only measured in experimental earthen ponds (Table 4.7). No major variation was observed in the value of water depth in two years of study. The highest mean depth was found 4.51±0.76 ft in $T_2$ (2014) whereas, the lowest water depth (4.22±0.69 ft) was recorded in $T_1$ in 2013 (Table 4.7).

**Table 4.7:** Water depth in various treatments of earthen ponds

| ***Year*** | ***Water depth in experimental ponds (ft)*** | | |
|---|---|---|---|
| | ***$T_1$*** | ***$T_2$*** | ***$T_3$*** |
| 2013 | 4.22±0.69 | 4.42±0.77 | 4.34±0.57 |
| 2014 | 4.50±0.78 | 4.51±0.76 | 4.36±0.77 |

*No statistically significant differences among treatments (p>0.05)*

Das and Kalita (2006) maintained a water depth of 40 cm in cement cistern in their study in Assam, India. Water depth is an important factor as the productivity of a water body depends on it. Water depth of 1.5 to 2.0 meters is recommended suitable for high productivity by Jhingran (1983) and DoF (2004).

### 4.6.4 pH

Water pH of the breeding environments, both aquaria and ponds, are shown in Figure 4.15 and 4.16. In case of breeding aquaria, the highest water pH was recorded 8.10 in $T_3$ in 2014. Whereas, the lowest water temperature was found 6.50 in $T_2$ in 2013. The mean water pH was 7.48 (Figure 4.15 and 4.16).

On the other hand, in ponds, the mean water pH was found 7.52 which was slightly higher than that of average pH in aquaria. The highest and lowest water pH was recorded 8.20 (in $T_2$ in 2014) and 6.50 (in $T_3$ in 2013) respectively (Figure 4.15 and 4.16).

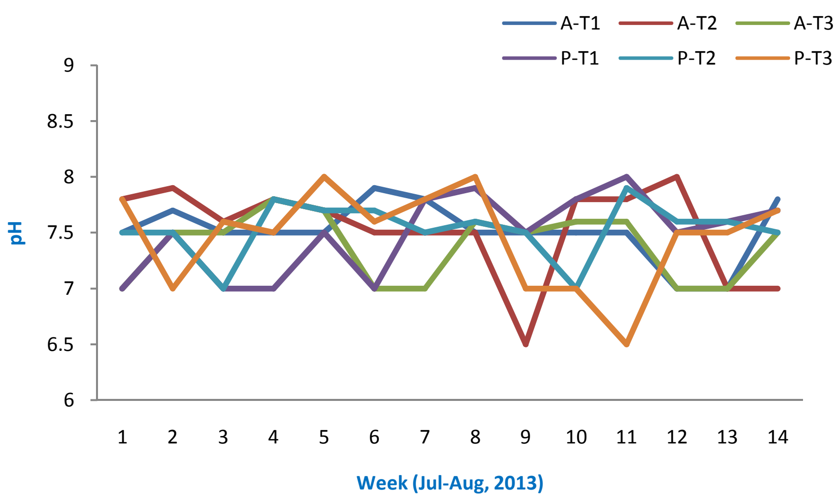



**Figure 4.15:** Water pH in breeding aquaria and ponds in 2013; A=Aquaria, P=Pond and T=Treatment

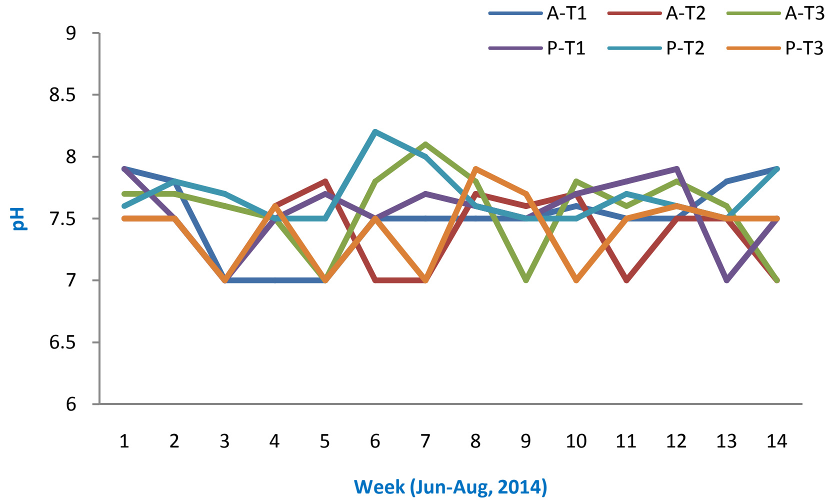



**Figure 4.16:** Water pH in breeding aquaria and ponds in 2014; A=Aquaria, P=Pond and T=Treatment

Table 4.8 shows the mean value of water pH in all the treatments, both experimental aquaria and earthen ponds. More or less similar pH level of water was observed in aquaria and ponds. The highest pH in aquaria was 7.57±0.34 (in $T_3$, 2014) and the lowest was 7.38±0.30 (in $T_3$, 2013). In case of ponds, the highest and lowest mean water pH was recorded 7.69±0.22 (in $T_2$, 2014) and 7.41±0.29 (in $T_3$, 2014) (Table 4.8).

**Table 4.8:** pH level in the breeding environment

| *Year* | *Aquaria* | | | *Experimental ponds* | | |
|---|---|---|---|---|---|---|
| | *$T_1$* | *$T_2$* | *$T_3$* | *$T_1$* | *$T_2$* | *$T_3$* |
| 2013 | 7.51±0.26 | 7.53±0.42 | 7.38±0.30 | 7.49±0.35 | 7.53±0.26 | 7.46±0.44 |
| 2014 | 7.50±0.31 | 7.39±0.31 | 7.57±0.34 | 7.56±0.28 | 7.69±0.22 | 7.41±0.29 |

*No statistically significant differences among treatments in both aquaria and earthen ponds in both years (p>0.05)*

Abujam *et al.* (2015) found that water pH level varied between 7.2 and 7.8 in a hormone induced breeding program of *T. fasciata* in India which was very similar to the findings of the present study. The spawning enhanced in acidic water with a pH range between 5.5 and 6.5 (Reyes-Bustamente and Ortega-Salas 2002). However, pH level recorded in the present research was quite same as natural wetlands of Bangladesh, for example the Meduary Beel of Mymensingh district (Chakraborty *et al.* 2012b).

In another research, Mohsin and Mondal (2013) reported that water pH level in aquarium varied from 7.13±0.05 to 7.47±0.07 during their work on survival of exotic ornamental fishes of Bangladesh which is quite similar to the present findings. Water pH ranging from 6.5 to 8.5 (at sunrise) are generally the most suitable for pond fish production (FAO 1997). According to DoF (2005) and Khaleque (2002) suitable range of pH is from 6.5 to 9.0 and 6.5 to 7.5 respectively. So, the pH recorded in the present research was in the suitable range.

### 4.6.5 Dissolved oxygen (DO)

Dissolved oxygen level of the breeding environments, both aquaria and ponds, are shown in Figure 4.17 and 4.18. In case of breeding aquaria, the highest DO pH was recorded 7.00 mg/l in $T_3$ in 2013. Whereas, the lowest DO value was found 4.25 mg/l in $T_1$ in 2013. The mean DO was 5.51 mg/l (Figure 4.17 and 4.18).

On the other hand, in ponds, the mean DO level was found 6.53 mg/l which was higher than that of average DO in aquaria. The highest and lowest water pH was recorded 8.00 mg/l (in $T_2$ in 2013) and 4.50 mg/l (in $T_1$ in 2014) respectively (Figure 4.17 and 4.18).

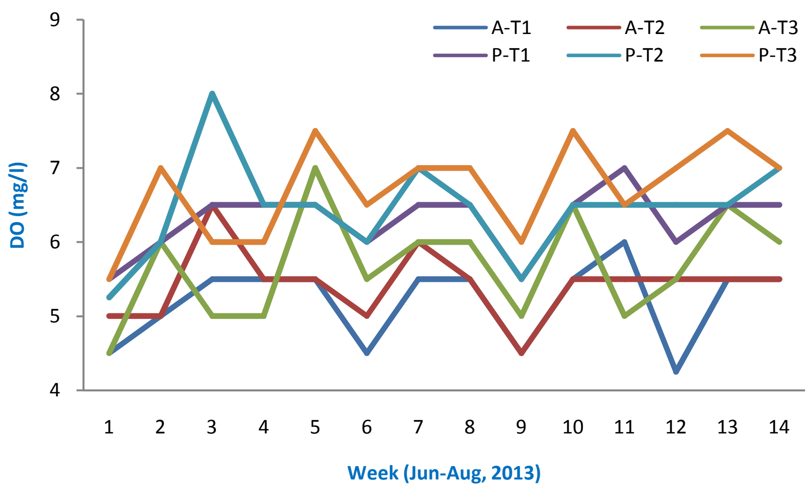



**Figure 4.17:** Dissolved oxygen level in breeding aquaria and ponds in 2013; A=Aquaria, P=Pond and T=Treatment

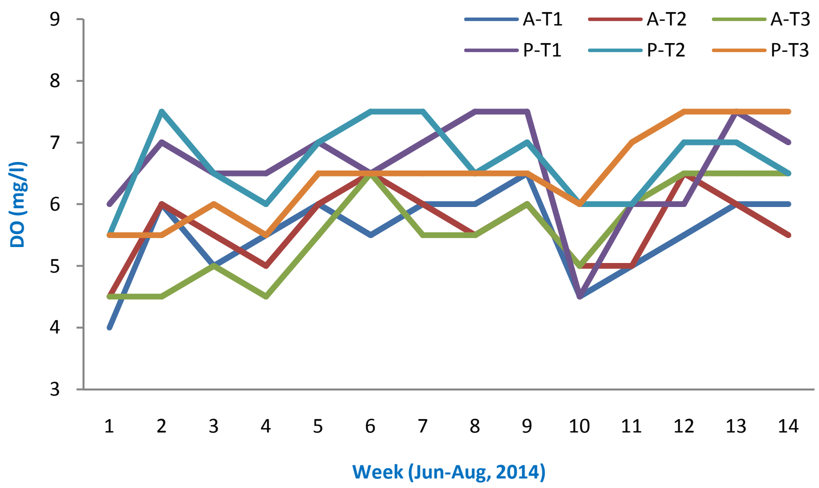



**Figure 4.18:** Dissolved oxygen level in breeding aquaria and ponds in 2014; A=Aquaria, P=Pond and T=Treatment

Table 4.9 shows the mean value of DO in all the treatments, both experimental aquaria and earthen ponds. Comparatively higher DO level of water was observed in the experimental ponds than that of aquaria. The highest DO in aquaria was 5.68±0.72 mg/l (in $T_3$, 2013) and the lowest was 5.19±0.54 mg/l (in $T_1$, 2013). In case of ponds, the highest and lowest mean DO was recorded 6.71±0.64 mg/l (in $T_3$, 2013) and 6.29±0.43 mg/l (in $T_1$, 2013) (Table 4.9).

**Table 4.9:** Dissolved oxygen (mg/l) in aquaria and ponds

| ***Year*** | ***Aquaria*** | | | ***Experimental ponds*** | | |
|---|---|---|---|---|---|---|
| | ***$T_1$*** | ***$T_2$*** | ***$T_3$*** | ***$T_1$*** | ***$T_2$*** | ***$T_3$*** |
| 2013 | 5.19±0.54 | 5.43±0.47 | 5.68±0.72 | 6.29±0.43 | 6.44±0.67 | 6.71±0.64 |
| 2014 | 5.54±0.69 | 5.64±0.60 | 5.57±0.78 | 6.61±0.81 | 6.68±0.64 | 6.46±0.72 |

*No statistically significant differences among treatments in both aquaria and earthen ponds in both years ($p>0.05$)*

Abujam *et al.* (2015) recorded a DO range of 5.12 to 8.06 mg/l in a breeding experiment of *T. fasciata* using ovaprim hormone in India where the breeding program was a successful one. The results recorded in the present study were also within this range and thus, the level of DO can be considered suitable for the breeding of gouramies. However, alkalinity recorded in the present research was higher than that of natural wetlands of Bangladesh, for example the Meduary Beel of Mymensingh district where DO range of 4.44 mg/l to 4.88 mg/l was reported by Chakraborty *et al.* (2012b). In another research, Mohsin and Mondal (2013) reported that DO level in aquarium varied from 3.80±0.06 to 4.73±0.07 mg/l during their work on survival of exotic ornamental fishes of Bangladesh which is lower than that of the present findings.

### 4.6.6 Free carbon dioxide ($CO_2$)

Free $CO_2$ level of the breeding environments, both aquaria and ponds, are shown in Figure 4.19 and 4.20. In case of breeding aquaria, the highest free $CO_2$ was recorded 10.50 mg/l in $T_2$ in 2013. Whereas, the lowest free $CO_2$ was found 5.00 mg/l in $T_3$ in 2013. The mean free $CO_2$ was 7.52 mg/l (Figure 4.19 and 4.20).

On the other hand, in ponds, the mean free $CO_2$ was found 8.81 mg/l which was slightly higher than that of average free $CO_2$ level in aquaria. The highest and lowest free $CO_2$ was recorded 11.50 mg/l (in $T_1$ in 2014) and 5.50 mg/l (in $T_3$ in 2014) respectively (Figure 4.19 and 4.20).

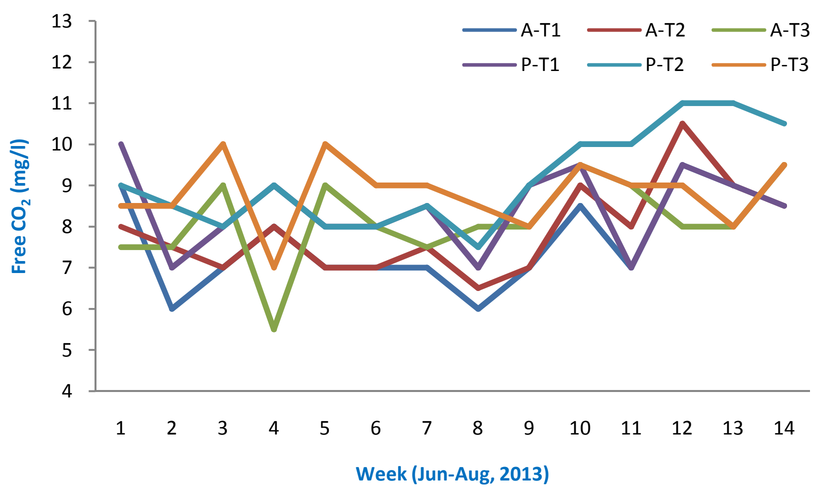



**Figure 4.19:** Free carbon dioxide level in breeding aquaria and ponds in 2013; A=Aquaria, P=Pond and T=Treatment

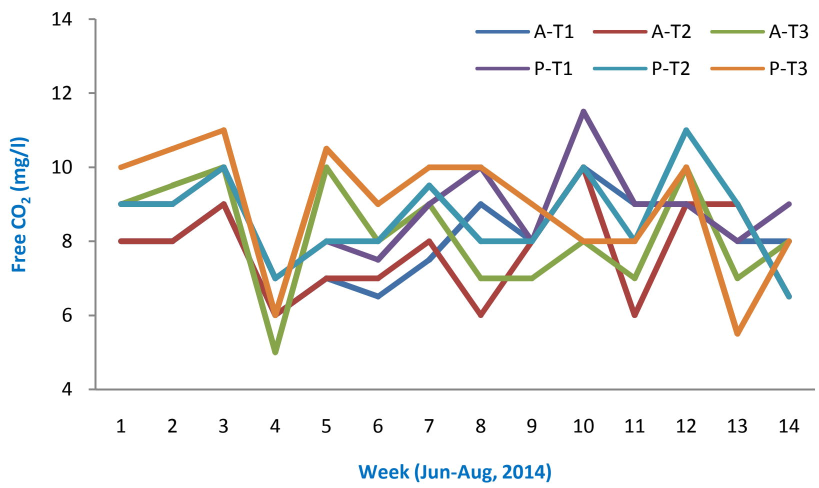



**Figure 4.20:** Free carbon dioxide level in breeding aquaria and ponds in 2014; A=Aquaria, P=Pond and T=Treatment

Table 4.10 shows the mean value of free $CO_2$ in all the treatments, both experimental aquaria and earthen ponds. Comparatively higher free $CO_2$ level of water was observed in the experimental ponds than that of aquaria. The highest free $CO_2$ in aquaria was 8.18±1.49 mg/l (in $T_3$, 2014) and the lowest was 7.61±1.13 mg/l (in $T_1$, 2013). In case of ponds, the highest and lowest mean free $CO_2$ was recorded 9.14±1.17 mg/l (in $T_2$, 2013) and 8.43±0.98 mg/l (in $T_1$, 2013) (Table 4.10).

**Table 4.10:** Mean free $CO_2$ (mg/l) in different treatments of breeding environments

| *Year* | *Aquaria* | | | *Experimental ponds* | | |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_1$ | $T_2$ | $T_3$ |
| 2013 | 7.61±1.13 | 7.89±1.08 | 8.14±1.05 | 8.43±0.98 | 9.14±1.17 | 8.82±0.82 |
| 2014 | 8.07±1.09 | 7.68±1.29 | 8.18±1.49 | 8.86±1.15 | 8.64±1.23 | 8.96±1.68 |

*No statistically significant differences among treatments in both aquaria and earthen ponds in both years (p>0.05)*

Abujam *et al.* (2015) conducted a breeding trial of banded gourami, *T. fasciata* using ovaprim hormone injection in India. They have found that the free $CO_2$ level in breeding program varied from 3.23 to 9.42 mg/l. The results recorded in the present study were within this range. In another research, Mohsin and Mondal (2013) reported that free $CO_2$ level in aquarium varied from 9.21±0.05 to 11.75±0.03 mg/l during their work on survival of exotic ornamental fishes of Bangladesh which is quite similar to the present findings.

### 4.6.7 Alkalinity

Alkalinity level in water of the breeding environments, both aquaria and ponds, are shown in Figure 4.21 and 4.22. In case of breeding aquaria, the highest alkalinity was recorded 175 mg/l in $T_3$ in 2014. Whereas, the lowest water alkalinity was found 45 mg/l in $T_1$ in 2013. The mean water alkalinity was 122.92 mg/l (Figure 4.21 and 4.22).

On the other hand, in ponds, the mean water alkalinity was found 110.71 mg/l which was slightly lower than that of average alkalinity level in aquaria. The highest and lowest alkalinity was recorded 175 mg/l (in $T_3$ in 2014) and 50 mg/l (in $T_1$ in 2013) respectively (Figure 4.21 and 4.22).

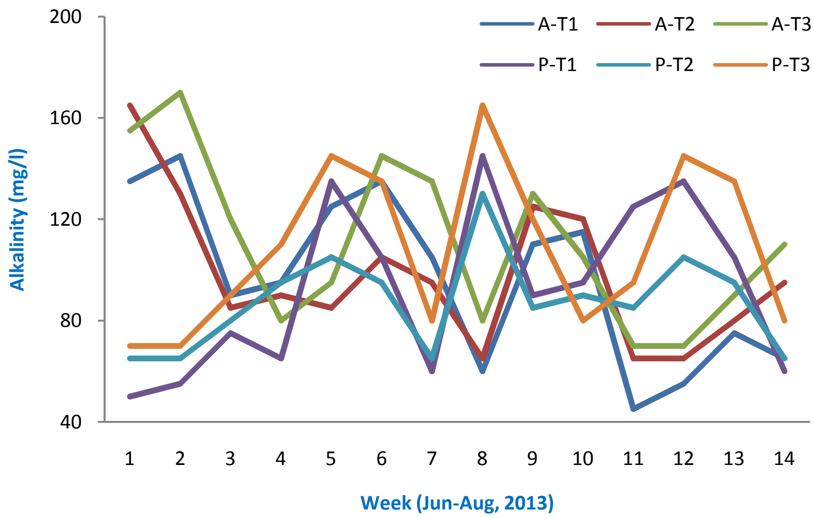



**Figure 4.21:** Alkalinity level in breeding aquaria and ponds in 2013; A=Aquaria, P=Pond and T=Treatment

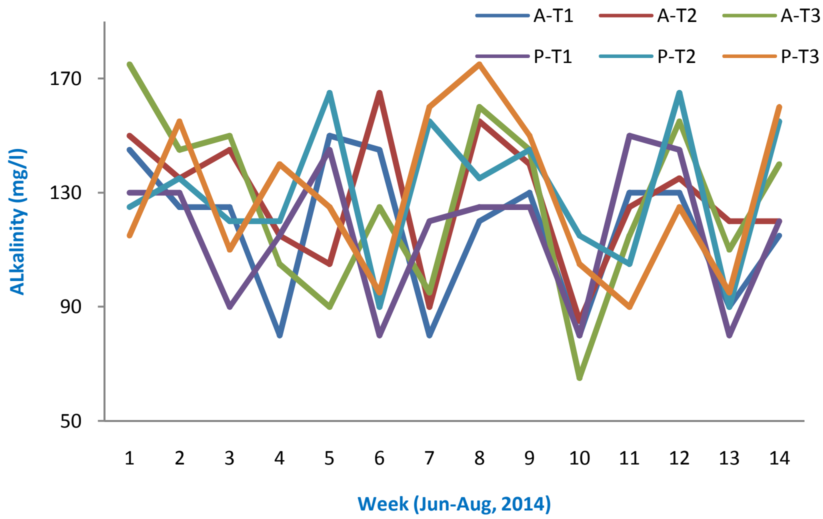



**Figure 4.22:** Alkalinity level in breeding aquaria and ponds in 2014; A=Aquaria, P=Pond and T=Treatment

Table 4.11 shows the mean value of alkalinity in all the treatments, both experimental aquaria and earthen ponds. More or less similar alkalinity level of water was observed in both experimental ponds and aquaria. The highest alkalinity in aquaria was 127.50±23.59 mg/l (in $T_2$, 2014) and the lowest was 96.79±32.73 mg/l (in $T_1$, 2013). In case of ponds, the highest and lowest mean alkalinity was recorded 130.00±25.19 mg/l (in $T_2$, 2014) and 87.50±18.99 mg/l (in $T_2$, 2013) (Table 4.11).

**Table 4.11:** Treatment-wise average alkalinity (mg/l) level in aquaria and earthen ponds

| *Year* | *Aquaria* | | | *Experimental ponds* | | |
|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_1$ | $T_2$ | $T_3$ |
| 2013 | 96.79±32.73$^{c}$ | 96.86±28.80$^{c}$ | 111.07±32.35$^{ab}$ | 92.86±32.98$^{c}$ | 87.50±18.99$^{c}$ | 108.57±32.01$^{ab}$ |
| 2014 | 117.50±25.01 | 127.50±23.59 | 126.79±31.23 | 116.78±24.78$^{b}$ | 130.00±25.19$^{a}$ | 128.57±28.11 |

***p = 0.05***
**a**, results significantly varies with $T_1$;
**b**, result significantly varies with $T_2$;
**c**, result significantly varies with $T_3$

Abujam *et al.* (2015) conducted a breeding trial of banded gourami, *T. fasciata* using ovaprim hormone injection in India. In that research the researchers have recorded that the alkalinity level varied between 20 mg/l and 49 mg/l which were much lower than that of alkalinity values recorded in present study. However, alkalinity recorded in the present research was quite same as natural wetlands of Bangladesh, for example the Meduary Beel of Mymensingh district where an alkalinity range of 115.55 mg/l to 127.42 mg/l was reported by Chakraborty *et al.* (2012b). Mohsin and Mondal (2013) reported that total alkalinity level in aquarium varied from 76.66±1.64 to 108.92±3.20 mg/l during their work on survival of exotic ornamental fishes of Bangladesh which is quite lower than that of the present findings. Water alkalinity of more than 100 mg/l is considered standard for aquatic habitats (Rahman 1992) so the alkalinity levels recorded in almost all the treatments of the present study were within the suitable range. Islam *et al.* (2012) recorded alkalinity range between 151.6 and 497 mg/l during their study in the Dhaleshwari River of Bangladesh which is quite high than that of the results of present study.

### 4.6.8 Ammonia-Nitrogen ($NH_3$-$N_2$)

Ammonia-Nitrogen level in water of aquaria, are shown in Figure 4.23 and 4.24. The highest $NH_3$-$N_2$ was recorded 0.0061 mg/l in $T_3$ in 2014. Whereas, the lowest $NH_3$-N was found 0.0011 mg/l in $T_1$ in 2013. The mean water $NH_3$-$N_2$ was 0.0026 mg/l (Figure 4.23 and 4.24).

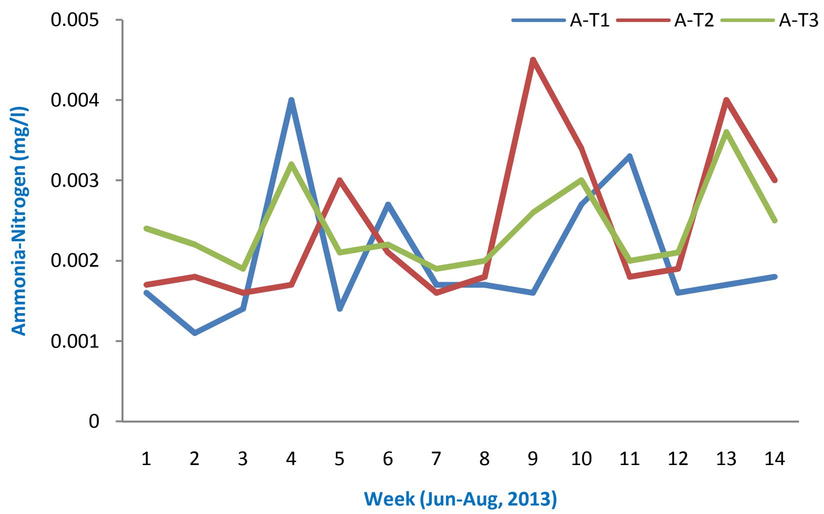



**Figure 4.23:** Ammonia-nitrogen level in breeding aquaria in 2013; A=Aquaria, P=Pond and T=Treatment

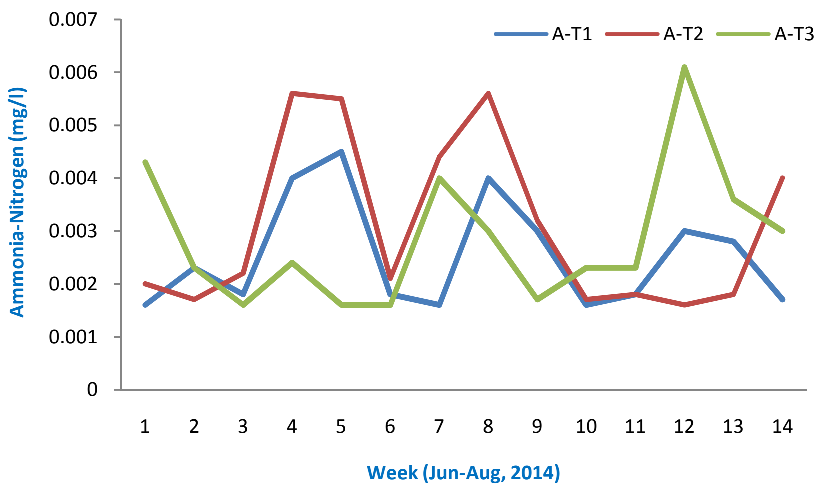



**Figure 4.24:** Ammonia-nitrogen level in breeding aquaria in 2014; A=Aquaria, P=Pond and T=Treatment

Ammonia was only measured in experimental aquaria (Table 4.12). No major variation was observed in the value of water ammonia in two years of captive breeding trials. The highest mean ammonia was found 0.003±0.0010 mg/l in $T_2$ (2014) (Table 4.12).

**Table 4.12:** Mean ammonia-nitrogen level in different treatments

| Year | Ammonia-nitrogen in aquaria (mg/l) | | |
|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ |
| 2013 | 0.002±0.0008 | 0.002±0.0009 | 0.002±0.0005 |
| 2014 | 0.002±0.0010 | 0.003±0.0010 | 0.002±0.0010 |

*No statistically significant differences among treatments (p>0.05)*

Modal (2012) recorded ammonia-nitrogen level from nil to 0.01 mg/l in experimental aquaria during his study on survival of ornamental fishes which is little higher than that of the found in the present research. Mohsin and Mondal (2013) reported that ammonia-nitrogen level in aquarium varied from 0.0010±0.0006to 0.0133±0.002 mg/l during their work on survival of exotic ornamental fishes of Bangladesh.

### 4.6.9 Chlorine

Chlorine level was measured in water of aquaria only, and the results are shown in Figure 4.25 and 4.26. The highest chlorine level was recorded 0.074 mg/l in $T_1$ in 2014. Whereas, the lowest chlorine level was found 0.007 mg/l in $T_1$ in 2013. The mean chlorine level in water was calculated 0.0279 mg/l (Figure 4.25 and 4.26).

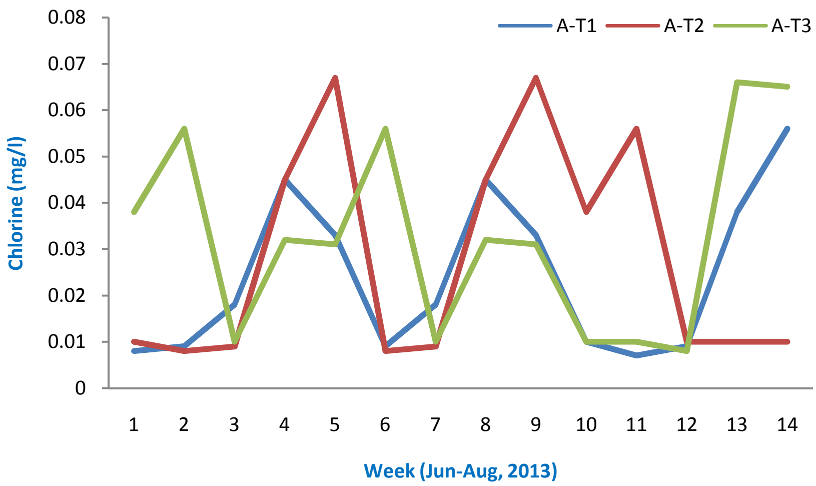



**Figure 4.25:** Chlorine level in breeding aquaria in 2013; A=Aquaria, P=Pond and T=Treatment

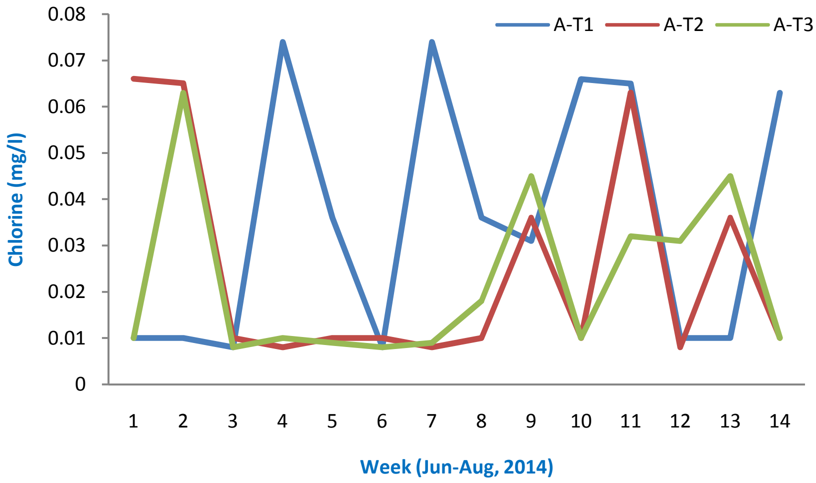



**Figure 4.26:** Chlorine level in breeding aquaria in 2014; A=Aquaria, P=Pond and T=Treatment

Chlorine level of water was also only measured in experimental aquaria (Table 4.13). No major variation was observed in the value of chlorine in two years of captive breeding trials. The highest mean depth was found 0.04±0.03 mg/l in $T_1$ (2014) (Table 4.13).

**Table 4.13:** Average chlorine level in the breeding environments

| ***Year*** | ***Chlorine level in aquaria (mg/l)*** | | |
|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ |
| 2013 | 0.02±0.02 | 0.03±0.02 | 0.03±0.02 |
| 2014 | 0.04±0.03 | 0.03±0.02 | 0.02±0.02 |

*No statistically significant differences among treatments (p>0.05)*

Modal (2012) recorded chlorine level from 0.003 to 0.016 mg/l in experimental aquaria during his study on survival of ornamental fishes which is little lower than that of the found in the present research. In another research, Mohsin and Mondal (2013) reported that chlorine level in aquarium varied from 0.0045±0.001 to 0.012±0.0014 mg/l during their work on survival of exotic ornamental fishes of Bangladesh. In a study by Yasmeen *et al.* (2012) chloride value of 12.75 to 30.65 mg/l was recorded in the Buriganga River of Bangladesh which is far greater than that of the present findings.

## 4.7 Relationships among water quality parameters and breeding performances

### 4.7.1 Relationship in experimental aquaria

Table 4.14 shows the correlation between water quality parameters and captive breeding performances in experimental aquaria. Highly significant correlation (0.954**) was found between production of giant/banded and dwarf gouramies. Positive correlation between breeding of both species of gouramies and water quality parameters were revealed from the Pearson correlation analysis expect for ammonia and chlorine (Table 4.14).

**Table 4.14:** Pearson correlation among breeding performances and water quality parameters in experimental aquaria

| | *Dwarf* | *Temp.* | *Free $CO_2$* | *Alkalinity* | *$NH_3$-$N_2$* | *Chlorine* | *pH* | *DO* |
|---|---|---|---|---|---|---|---|---|
| ***Giant*** | 0.954** | 0.294 | 0.564 | 0.168 | -0.598 | -0.136 | 0.217 | 0.008 |
| ***Dwarf*** | | 0.191 | 0.574 | 0.162 | -0.533 | -0.216 | 0.012 | 0.127 |
| ***Temp.*** | | | 0.224 | 0.420 | -0.103 | -0.604 | 0.700 | -0.089 |
| ***Free $CO_2$*** | | | | 0.353 | -0.503 | 0.229 | 0.162 | 0.593 |
| ***Alkalinity*** | | | | | 0.525 | 0.129 | -0.202 | 0.733 |
| ***$NH_3$-$N_2$*** | | | | | | 0.108 | -0.569 | 0.362 |
| ***Chlorine*** | | | | | | | -0.343 | 0.429 |
| ***pH*** | | | | | | | | -0.525 |

*** Correlation is significant at the 0.01 level (2-tailed)*

### 4.7.2 Relationship in Experimental earthen ponds

Table 4.15 shows the correlation between water quality parameters and captive breeding performances in experimental earthen ponds. Highly significant correlation (0.979**) was found between production of giant/banded and dwarf gouramies. Negative correlation between breeding of both species of gouramies and water quality parameters were revealed from the Pearson correlation analysis expect for alkalinity and water temperature (Table 4.15).

**Table 4.15:** Pearson correlation among breeding performances and water quality parameters in experimental earthen ponds

| | *Dwarf* | *Temp.* | *Transpa.* | *Depth* | *pH* | *DO* | *Free $CO_2$* | *Alkalinity* |
|---|---|---|---|---|---|---|---|---|
| ***Giant*** | 0.979** | 0.356 | -0.274 | -0.434 | -0.468 | -0.137 | -0.479 | 0.084 |
| ***Dwarf*** | | 0.453 | -0.262 | -0.356 | -0.407 | -0.099 | -0.529 | 0.263 |
| ***Temp.*** | | | 0.441 | -0.471 | -0.670 | -0.288 | -0.216 | 0.409 |
| ***Transpa.*** | | | | -0.452 | -0.396 | -0.838* | 0.109 | -0.344 |
| ***Depth*** | | | | | 0.688 | 0.654 | 0.443 | 0.502 |
| ***pH*** | | | | | | 0.404 | -0.155 | 0.230 |
| ***DO*** | | | | | | | 0.198 | 0.561 |
| ***$CO_2$*** | | | | | | | | -0.140 |

No previous studies were found where relationship between breeding performances and water quality parameters were established. Thus, it was not possible to compare the results of the present research against a previous one. This problem is not a new one and also mentioned by Chaki *et al.* (2014).

However, in research by Chaki *et al.* (2014) relationship between water quality parameters and seasonal fish abundance in the Atrai River was studied. The research findings indicated that environmental parameters of aquatic habitats were closely correlated with the availability of fish species. Correlation among physico-chemical variables in nursery, growout and broodstock ponds was studied by Hossain *et al.* (2013) and the results revealed the strong correlation among different physical and chemical factors.

## 4.8 Breeding behavior

Studying the behavior of the studied species, mainly in the experimental aquaria, following description of natural breeding can be mentioned. Both species of *Trichogaster* are territorial and nesting species. The males have a higher differentiated behavior towards males and females of the same species. At the onset of the spawning cycle, a male constructs a bubble nest at the air-water surface with bubble and usually with a bit of vegetation (water velvet, *Pistia* sp., in experimental aquaria).

When ready to spawn, the male courts the female with mild aggression. A male that was ready to spawn, usually allowed a receptive female to enter the territory or dashed out to a nearby female led her to the next. After a period of courtship, a pair spawned directly beneath the nest. The majority of the eggs float into the bubble nest. The act was repeated until the female is spent and then she permanently left the nest.

The male retrieved the fallen eggs and carried them to the nest in his mouth. After that the male guards the nest and the surrounding territory. The eggs hatched and the larvae remained in the nest until they became free swimming and absorb the yolk sac. After the juvenile dispersed, the male left the nest and offspring.

The present findings are similar to the findings of several other researchers. According to Degani (1989) and Axelrod (1980), the male of *Trichogaster* sp. builds a floating bubble nest in which the eggs are laid and other bubble nest builders, males will incorporate bits of plants, twigs, and other debris, which hold the nest together better. Das (2003) reported that the *Colisa* species are known to breed in a little weed infested water tank.

## 4.9 Life cycle

An outline of the simplest form of life cycle of gouramies is shown in Figure 4.27. The studied species, especially those are collected from nature for breeding, were avoidant in nature and thus required quite or covered aquaria for breeding. Male constructed a bubble nest in which female laid eggs followed by release of sperms by the male. Hatching took place between 18 to 24 hours from fertilized eggs. In aquaria, plastic containers or other similar substances acted perfectly as breeding substances.

Newly born larvae with a large yolk sac is inactive in the first phase. Later, yolk sac gradually reduced and larvae become more active. After several days, usually 3 to 5, the fry become free swimming. Both of the studied species, *T. fasciata* and *T. lalius*, attain sexual maturity at the age of 1 year.

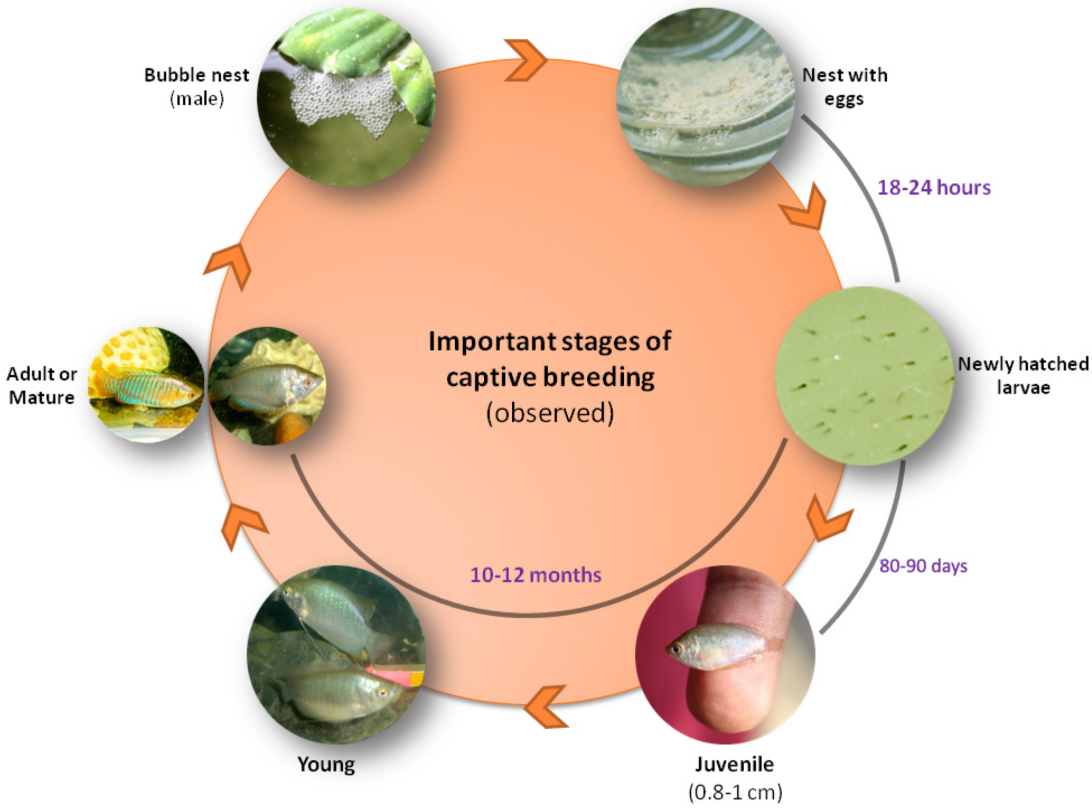



**Figure 4.27:** Some important stages of the life cycle of gouramies

## 4.10 Market potentialities and benefit-cost ratio (BCR)

An outline of BCR calculated for the gourami production in experimental aquaria and earthen ponds are shown in Table 4.16 and 4.17. Sample young produced in this research were sold to the aquarists with the help of a local aquarium fish trader and demand for these two species was found satisfactory (Figure 4.28, 4.29 and 4.30). All the individuals supplied to this ornamental fish outlet were sold within 2-5 days of supply. Retail price was good enough, between BDT 30 and 90 per pair depending on size and coloration of the fish individuals. The results represent the potentiality of *Trichogaster* species if sold as ornamental fish in the aquarium shops of Bangladesh.

### 4.10.1 BCR in aquaria

It was revealed from the research that BCR was found the highest in $T_1$ in 2014 which was 7.05±0.97. However, negative BCR was also recorded in some cases, especially in $T_2$ where studied species were treated with PG hormone administration (Table 4.16).

**Table 4.16:** Benefit-cost ratio of gourami production in aquaria

| ***Fish*** | ***Year*** | ***BCR ratio: [Mean±SD(SE)]*** | | |
|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_3$ |
| Giant gourami | 2013 | 1.14±1.00 (0.58)$^{b}$ | -1.00±00.00 (0.00)$^{a,c}$ | 1.27±0.56 (0.32)$^{b}$ |
| | 2014 | 7.05±0.97 (0.56)$^{b}$ | -0.50±0.50 (0.29)$^{a,c}$ | 5.90±1.00 (0.58)$^{b}$ |
| Dwarf gourami | 2013 | -0.14±0.19 (0.12)$^{b}$ | -1.00±00.00 (0.00)$^{a,c}$ | 0.18±0.17 (0.10)$^{b}$ |
| | 2014 | 1.63±0.35 (0.20)$^{b}$ | -0.49±0.50 (0.29)$^{a,c}$ | 1.77±0.31 (0.10)$^{b}$ |

***p=0.05***.
**a**, results significantly varies with $T_1$;
**b**, result significantly varies with $T_2$;
**c**, result significantly varies with $T_3$

### 4.10.2 BCR in earthen ponds

Two treatments ($T_1$ and $T_3$) were found highly beneficial in terms of earning profit from the culture. It was revealed from the research that BCR was found the highest in $T_1$ in 2014 which was 16.02±2.77. However, negative BCR was also recorded in some cases, especially in $T_2$ where studied species were treated with PG hormone administration (Table 4.17).

**Table 4.17:** Benefit-cost ratio of gourami production in earthen pond

| *Fish* | *Year* | *BCR ratio: [Mean±SD(SE)]* | | |
|---|---|---|---|---|
| | | $T_1$ | $T_2$ | $T_3$ |
| Giant gourami | 2013 | 15.52±2.85 (1.64)$^{b,c}$ | -1.00±00.00 (0.00)$^{a,c}$ | 10.57±0.74 (0.42)$^{a,b}$ |
| | 2014 | 16.02±2.77 (1.60)$^{b,c}$ | 1.87±0.42 (0.24)$^{a,c}$ | 9.87±1.10 (0.64)$^{a,b}$ |
| Dwarf gourami | 2013 | 4.53±1.18 (0.68)$^{b}$ | -1.00±00.00 (0.00)$^{a,c}$ | 2.76±1.04 (0.60)$^{b}$ |
| | 2014 | 4.89±0.87 (0.50)$^{b}$ | 0.95±0.53 (0.31)$^{a,c}$ | 3.40±0.47 (0.27)$^{b}$ |

***p=0.05***.
**a**, results significantly varies with $T_1$;
**b**, result significantly varies with $T_2$;
**c**, result significantly varies with $T_3$

**Figure 4.28:** Harvested gouramies from experimental ponds produced through captive breeding under the present study


**Figure 4.29:** Harvested young gouramies in plastic bag for marketing

**Figure 4.30:** Gouramies, produced in the present research, in an aquarium of a local ornamental fish outlet for selling

No previous studies to analyze the BCR or CBR (cost benefit ratio) of gouramies were found. But there are some researcher efforts conducted to assess the CBR or BCR based on some other aquaculture system, especially when cultured species were used as food fish. Mohsin *et al.* (2012) studied the CBR of carp polyculture in Natore and Rajshahi district of Bangladesh and found that CBR of this culture practice was 1:1.05 which is far lower than that of benefit calculated in the present research. Islam *et al.* (2008) recorded CBR of 1:1.54 in case of carp growout ponds. The recorded average net profit was found much higher than that of the findings of Alam *et al.* (1995) and Awal *et al.* (2001) who have reported CBR of 3.83 to 3.62 and 1:2.73 respectively.

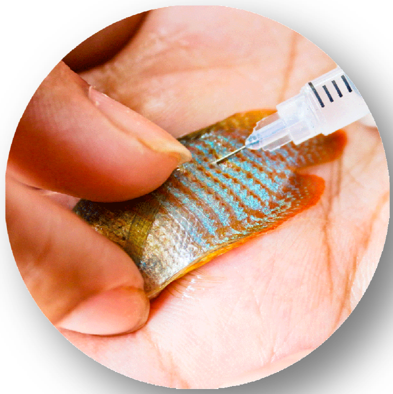
Retail price of the fishes produced in this research was found much higher because of changes in the type of retail market. Traditionally, gouramies are used as food fish in Bangladesh and retail price of this fish was reported BDT 30 to 49 per kg, which contains over one hundred individuals (Galib 2008) whereas if the fishes are sold in aquarium markets, price of one a pair could bring double profit.

The breeding technology of gouramies in the present research could particularly be useful for both the conservation of rapidly declining gourami species (*T. fasciata* and

*T. lalius*) and also for the expansion of ornamental trade of Bangladesh, both inside and outside country. Captive breeding carried out in aquaria can be more suitable in urban areas where sufficient land area may not be found for the construction of ponds. On the other hand, interested people can use earthen ponds in rural areas to carry out this breeding in captivity as availability of land is more in rural areas.


## CHAPTER FIVE

# CONCLUSION



# CONCLUSION

Based on the research questions and research findings of the present study following conclusion can be drawn- young or juvenile gouramies collected from wild sources can easily adopt and spawn in captive environment; both of the gourami species breed in captivity with or without inducing hormone administration but quality of inducing hormone need to be ensured; all water quality parameters were in suitable range for aquatic organisms and did not adversely affect the breeding programs; the survival of newly born offspring is quite good and satisfactory; and finally there is a good potential market for studied species in Bangladesh and if supplied to the aquarium fish outlets, aquarist or ornamental fish keepers would accept both of the species.

Performance of PG hormone was too poor and thus could not be recommended for mass-scale production of offspring. However, further research is also recommended to assess the breeding performance of gouramies at different doses of PG hormone. The breeding technology of gouramies in the present research could particularly be useful for both the conservation of rapidly declining gourami species (*T. fasciata* and *T. lalius*) and also for the expansion of ornamental trade of Bangladesh, both inside and outside country.

Captive breeding carried out in aquaria can be more suitable in urban areas where sufficient land area may not be found for the construction of ponds. On the other hand, interested people can use earthen ponds in rural areas to carry out this breeding in captivity as availability of land is more in rural areas. Benefit of these two gourami species could be remarkable if sold as ornamental fish apart from traditional use as food fish. As the studied species easily breed in captivity, hormone administration could be avoided to reduce the cost of production.

Both of the gouramies are of avoidant nature and prefer to hide in the dark or shady places among aquatic vegetations during daylight and they use these aquatic plants as their breeding ground. So, sufficient aquatic vegetations need be ensured in the breeding environment for successful breeding. Breeding in aquaria set to direct exposure of sunlight would give maximum breeding performance as this helps to create an environmental inside the aquarium similar to that of wild. However, gourami species can also breed in aquaria in an indoor environment but survival of newly born offspring may not be satisfactory. However, further research can also be conducted on the improvement of survival rate of gourami larvae in aquaria placed in an indoor environment.

Results of the present research have revealed the prospects of banded/giant and dwarf gouramies in Bangladesh and would inspire interested researchers to conduct researches based on gourami species.

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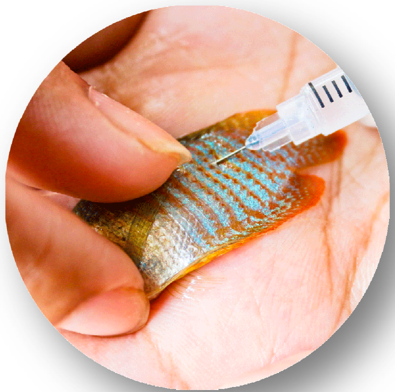

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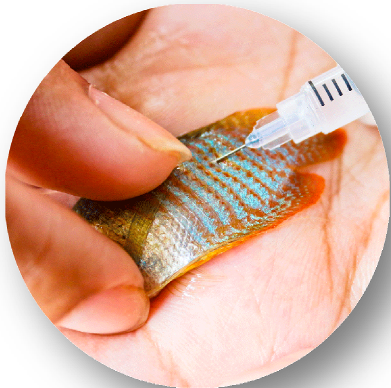
# APPENDICES



# APPENDICES

## Appendix 1: Water quality data

**Appendix Table 1:** Water temperature in 2013

| Week No. | $A\text{-}T_1$ | $A\text{-}T_2$ | $A\text{-}T_3$ | $P\text{-}T_1$ | $P\text{-}T_2$ | $P\text{-}T_3$ |
|---|---|---|---|---|---|---|
| 1 | 31 | 29 | 29 | 33 | 33 | 34 |
| 2 | 29 | 29 | 28 | 33 | 33 | 32 |
| 3 | 29.5 | 29 | 29 | 34 | 33.5 | 33 |
| 4 | 26 | 26 | 27 | 30 | 30 | 31 |
| 5 | 28.5 | 29 | 30.5 | 32.5 | 33 | 34.5 |
| 6 | 28 | 26 | 26 | 32 | 30 | 30 |
| 7 | 26 | 26 | 24 | 30 | 30 | 28.5 |
| 8 | 28.5 | 28.5 | 28 | 32.5 | 32 | 32 |
| 9 | 28.5 | 29 | 29 | 32.5 | 33 | 32 |
| 10 | 28 | 28 | 27.5 | 32 | 32 | 31.5 |
| 11 | 28 | 28 | 27 | 30.5 | 30.5 | 31 |
| 12 | 27 | 28 | 28.5 | 31 | 30 | 30 |
| 13 | 27 | 28 | 26 | 29.5 | 30 | 30.5 |
| 14 | 26 | 26 | 27 | 29 | 29 | 31 |
| *Mean* | *27.92857* | *27.82143* | *27.60714* | *31.53571* | *31.35714* | *31.5* |
| *Max* | *31* | *29* | *30.5* | *34* | *33.5* | *34.5* |
| *Min* | *26* | *26* | *24* | *29* | *29* | *28.5* |
| *SD* | *1.439246* | *1.26502* | *1.63117* | *1.524975* | *1.561909* | *1.60528* |

*A, Aquarium; P, Pond; T, Treatment*

**Appendix Table 2:** Water temperature in 2014

| Week No. | A-$T_1$ | A-$T_2$ | A-$T_3$ | P-$T_1$ | P-$T_2$ | P-$T_3$ |
|---|---|---|---|---|---|---|
| 1 | 30 | 28.5 | 28.5 | 34 | 32.5 | 32.5 |
| 2 | 28 | 28 | 29 | 32 | 32 | 33 |
| 3 | 28 | 28 | 27.5 | 32 | 33 | 31.5 |
| 4 | 27 | 27 | 28.5 | 31 | 31 | 32.5 |
| 5 | 28.5 | 29 | 30 | 32.5 | 33 | 34 |
| 6 | 28 | 27 | 29 | 30 | 31 | 33 |
| 7 | 25 | 25 | 26.5 | 29 | 29.5 | 30.5 |
| 8 | 29 | 29.5 | 29 | 33 | 33.5 | 33 |
| 9 | 29 | 29 | 30 | 33 | 32 | 33 |
| 10 | 28 | 28 | 28.5 | 32 | 31 | 31 |
| 11 | 28 | 28 | 29 | 31 | 31 | 30 |
| 12 | 28 | 28 | 29 | 30 | 30 | 29 |
| 13 | 27 | 27 | 26.5 | 30 | 30 | 31 |
| 14 | 26 | 28 | 28 | 30 | 30.5 | 31 |
| *Mean* | 27.82143 | 27.85714 | 28.5 | 31.39286 | 31.42857 | 31.78571 |
| *Max* | 30 | 29.5 | 30 | 34 | 33.5 | 34 |
| *Min* | 25 | 25 | 26.5 | 29 | 29.5 | 29 |
| *SD* | 1.26502 | 1.116805 | 1.074172 | 1.469937 | 1.253566 | 1.423893 |

*A, Aquarium; P, Pond; T, Treatment*

**Appendix Table 3:** Water transparency in 2013 and 2014

| Week No. | 2013 | | | 2014 | | |
|---|---|---|---|---|---|---|
| | P-$T_1$ | P-$T_2$ | P-$T_3$ | P-$T_1$ | P-$T_2$ | P-$T_3$ |
| 1 | 30 | 32 | 31 | 32 | 31 | 31 |
| 2 | 31 | 31 | 36 | 33 | 31 | 33 |
| 3 | 33 | 31 | 30 | 37 | 32 | 29 |
| 4 | 30 | 34 | 31 | 34 | 35 | 35 |
| 5 | 31 | 31 | 32 | 30 | 31 | 36 |
| 6 | 37 | 34 | 33 | 30 | 41 | 39 |
| 7 | 31 | 45 | 34 | 39 | 33 | 32 |
| 8 | 33 | 33 | 31 | 31 | 32 | 38 |
| 9 | 38 | 35 | 32 | 32 | 30 | 33 |
| 10 | 36 | 40 | 33 | 32 | 42 | 37 |
| 11 | 33 | 33 | 38 | 30 | 31 | 36 |
| 12 | 41 | 31 | 30 | 30 | 32 | 35 |
| 13 | 44 | 41 | 31 | 31 | 31 | 31 |
| 14 | 32 | 33 | 32 | 33 | 30 | 40 |
| *Mean* | 34.28571 | 34.57143 | 32.42857 | 32.42857 | 33 | 34.64286 |
| *Max* | 44 | 45 | 38 | 39 | 42 | 40 |
| *Min* | 30 | 31 | 30 | 30 | 30 | 29 |
| *SD* | 4.322189 | 4.345011 | 2.277458 | 2.709365 | 3.823007 | 3.272429 |

*P, Pond; T, Treatment*

**Appendix Table 4:** Water pH in 2013

| *Week No.* | *A-$T_1$* | *A-$T_2$* | *A-$T_3$* | *P-$T_1$* | *P-$T_2$* | *P-$T_3$* |
|---|---|---|---|---|---|---|
| 1 | 7.5 | 7.8 | 7 | 7 | 7.5 | 7.8 |
| 2 | 7.7 | 7.9 | 7.5 | 7.5 | 7.5 | 7 |
| 3 | 7.5 | 7.6 | 7.5 | 7 | 7 | 7.6 |
| 4 | 7.5 | 7.8 | 7.8 | 7 | 7.8 | 7.5 |
| 5 | 7.5 | 7.7 | 7.7 | 7.5 | 7.7 | 8 |
| 6 | 7.9 | 7.5 | 7 | 7 | 7.7 | 7.6 |
| 7 | 7.8 | 7.5 | 7 | 7.8 | 7.5 | 7.8 |
| 8 | 7.5 | 7.5 | 7.6 | 7.9 | 7.6 | 8 |
| 9 | 7.5 | 6.5 | 7.5 | 7.5 | 7.5 | 7 |
| 10 | 7.5 | 7.8 | 7.6 | 7.8 | 7 | 7 |
| 11 | 7.5 | 7.8 | 7.6 | 8 | 7.9 | 6.5 |
| 12 | 7 | 8 | 7 | 7.5 | 7.6 | 7.5 |
| 13 | 7 | 7 | 7 | 7.6 | 7.6 | 7.5 |
| 14 | 7.8 | 7 | 7.5 | 7.7 | 7.5 | 7.7 |
| *Mean* | 7.514286 | 7.528571 | 7.378571 | 7.485714 | 7.528571 | 7.464286 |
| *Max* | 7.9 | 8 | 7.8 | 8 | 7.9 | 8 |
| *Min* | 7 | 6.5 | 7 | 7 | 7 | 6.5 |
| *SD* | 0.259755 | 0.421405 | 0.304274 | 0.354872 | 0.255489 | 0.436079 |

*A, Aquarium; P, Pond; T, Treatment*

**Appendix Table 5:** Water pH in 2014

| *Week No.* | *A-$T_1$* | *A-$T_2$* | *A-$T_3$* | *P-$T_1$* | *P-$T_2$* | *P-$T_3$* |
|---|---|---|---|---|---|---|
| 1 | 7.9 | 7.5 | 7.7 | 7.9 | 7.6 | 7.5 |
| 2 | 7.8 | 7.5 | 7.7 | 7.5 | 7.8 | 7.5 |
| 3 | 7 | 7 | 7.6 | 7 | 7.7 | 7 |
| 4 | 7 | 7.6 | 7.5 | 7.5 | 7.5 | 7.6 |
| 5 | 7 | 7.8 | 7 | 7.7 | 7.5 | 7 |
| 6 | 7.5 | 7 | 7.8 | 7.5 | 8.2 | 7.5 |
| 7 | 7.5 | 7 | 8.1 | 7.7 | 8 | 7 |
| 8 | 7.5 | 7.7 | 7.8 | 7.6 | 7.6 | 7.9 |
| 9 | 7.5 | 7.6 | 7 | 7.5 | 7.5 | 7.7 |
| 10 | 7.6 | 7.7 | 7.8 | 7.7 | 7.5 | 7 |
| 11 | 7.5 | 7 | 7.6 | 7.8 | 7.7 | 7.5 |
| 12 | 7.5 | 7.5 | 7.8 | 7.9 | 7.6 | 7.6 |
| 13 | 7.8 | 7.5 | 7.6 | 7 | 7.5 | 7.5 |
| 14 | 7.9 | 7 | 7 | 7.5 | 7.9 | 7.5 |
| *Mean* | 7.5 | 7.385714 | 7.571429 | 7.557143 | 7.685714 | 7.414286 |
| *Max* | 7.9 | 7.8 | 8.1 | 7.9 | 8.2 | 7.9 |
| *Min* | 7 | 7 | 7 | 7 | 7.5 | 7 |
| *SD* | 0.311325 | 0.310972 | 0.340652 | 0.276557 | 0.217882 | 0.293145 |

*A, Aquarium; P, Pond; T, Treatment*

**Appendix Table 6:** Dissolved oxygen level in 2013

| *Week No.* | *A-$T_1$* | *A-$T_2$* | *A-$T_3$* | *P-$T_1$* | *P-$T_2$* | *P-$T_3$* |
|---|---|---|---|---|---|---|
| 1 | 4.5 | 5 | 4.5 | 5.5 | 5.25 | 5.5 |
| 2 | 5 | 5 | 6 | 6 | 6 | 7 |
| 3 | 5.5 | 6.5 | 5 | 6.5 | 8 | 6 |
| 4 | 5.5 | 5.5 | 5 | 6.5 | 6.5 | 6 |
| 5 | 5.5 | 5.5 | 7 | 6.5 | 6.5 | 7.5 |
| 6 | 4.5 | 5 | 5.5 | 6 | 6 | 6.5 |
| 7 | 5.5 | 6 | 6 | 6.5 | 7 | 7 |
| 8 | 5.5 | 5.5 | 6 | 6.5 | 6.5 | 7 |
| 9 | 4.5 | 4.5 | 5 | 5.5 | 5.5 | 6 |
| 10 | 5.5 | 5.5 | 6.5 | 6.5 | 6.5 | 7.5 |
| 11 | 6 | 5.5 | 5 | 7 | 6.5 | 6.5 |
| 12 | 4.25 | 5.5 | 5.5 | 6 | 6.5 | 7 |
| 13 | 5.5 | 5.5 | 6.5 | 6.5 | 6.5 | 7.5 |
| 14 | 5.5 | 5.5 | 6 | 6.5 | 7 | 7 |
| *Mean* | 5.196429 | 5.428571 | 5.678571 | 6.285714 | 6.446429 | 6.714286 |
| *Max* | 6 | 6.5 | 7 | 7 | 8 | 7.5 |
| *Min* | 4.25 | 4.5 | 4.5 | 5.5 | 5.25 | 5.5 |
| *SD* | 0.538682 | 0.474631 | 0.72343 | 0.425815 | 0.666352 | 0.641941 |

*A, Aquarium; P, Pond; T, Treatment*

**Appendix Table 7:** Dissolved oxygen level in 2014

| *Week No.* | *A-$T_1$* | *A-$T_2$* | *A-$T_3$* | *P-$T_1$* | *P-$T_2$* | *P-$T_3$* |
|---|---|---|---|---|---|---|
| 1 | 4 | 4.5 | 4.5 | 6 | 5.5 | 5.5 |
| 2 | 6 | 6 | 4.5 | 7 | 7.5 | 5.5 |
| 3 | 5 | 5.5 | 5 | 6.5 | 6.5 | 6 |
| 4 | 5.5 | 5 | 4.5 | 6.5 | 6 | 5.5 |
| 5 | 6 | 6 | 5.5 | 7 | 7 | 6.5 |
| 6 | 5.5 | 6.5 | 6.5 | 6.5 | 7.5 | 6.5 |
| 7 | 6 | 6 | 5.5 | 7 | 7.5 | 6.5 |
| 8 | 6 | 5.5 | 5.5 | 7.5 | 6.5 | 6.5 |
| 9 | 6.5 | 6 | 6 | 7.5 | 7 | 6.5 |
| 10 | 4.5 | 5 | 5 | 4.5 | 6 | 6 |
| 11 | 5 | 5 | 6 | 6 | 6 | 7 |
| 12 | 5.5 | 6.5 | 6.5 | 6 | 7 | 7.5 |
| 13 | 6 | 6 | 6.5 | 7.5 | 7 | 7.5 |
| 14 | 6 | 5.5 | 6.5 | 7 | 6.5 | 7.5 |
| *Mean* | 5.535714 | 5.642857 | 5.571429 | 6.607143 | 6.678571 | 6.464286 |
| *Max* | 6.5 | 6.5 | 6.5 | 7.5 | 7.5 | 7.5 |
| *Min* | 4 | 4.5 | 4.5 | 4.5 | 5.5 | 5.5 |
| *SD* | 0.692384 | 0.602194 | 0.780955 | 0.812843 | 0.638723 | 0.719623 |

*A, Aquarium; P, Pond; T, Treatment*

**Appendix Table 8:** Free $CO_2$ level in 2013

| *Week No.* | *A-T₁* | *A-T₂* | *A-T₃* | *P-T₁* | *P-T₂* | *P-T₃* |
|---|---|---|---|---|---|---|
| 1 | 9 | 8 | 7.5 | 10 | 9 | 8.5 |
| 2 | 6 | 7.5 | 7.5 | 7 | 8.5 | 8.5 |
| 3 | 7 | 7 | 9 | 8 | 8 | 10 |
| 4 | 8 | 8 | 5.5 | 9 | 9 | 7 |
| 5 | 7 | 7 | 9 | 8 | 8 | 10 |
| 6 | 7 | 7 | 8 | 8 | 8 | 9 |
| 7 | 7 | 7.5 | 7.5 | 8.5 | 8.5 | 9 |
| 8 | 6 | 6.5 | 8 | 7 | 7.5 | 8.5 |
| 9 | 7 | 7 | 8 | 9 | 9 | 8 |
| 10 | 8.5 | 9 | 9.5 | 9.5 | 10 | 9.5 |
| 11 | 7 | 8 | 9 | 7 | 10 | 9 |
| 12 | 9.5 | 10.5 | 8 | 9.5 | 11 | 9 |
| 13 | 9 | 9 | 8 | 9 | 11 | 8 |
| 14 | 8.5 | 8.5 | 9.5 | 8.5 | 10.5 | 9.5 |
| *Mean* | 7.607143 | 7.892857 | 8.142857 | 8.428571 | 9.142857 | 8.821429 |
| *Max* | 9.5 | 10.5 | 9.5 | 10 | 11 | 10 |
| *Min* | 6 | 6.5 | 5.5 | 7 | 7.5 | 7 |
| *SD* | 1.129645 | 1.077365 | 1.045661 | 0.977775 | 1.167321 | 0.82292 |

*A, Aquarium; P, Pond; T, Treatment*

**Appendix Table 9:** Free $CO_2$ level in 2014

| *Week No.* | *A-T₁* | *A-T₂* | *A-T₃* | *P-T₁* | *P-T₂* | *P-T₃* |
|---|---|---|---|---|---|---|
| 1 | 8 | 8 | 9 | 9 | 9 | 10 |
| 2 | 8 | 8 | 9.5 | 9 | 9 | 10.5 |
| 3 | 9 | 9 | 10 | 10 | 10 | 11 |
| 4 | 6 | 6 | 5 | 7 | 7 | 6 |
| 5 | 7 | 7 | 10 | 8 | 8 | 10.5 |
| 6 | 6.5 | 7 | 8 | 7.5 | 8 | 9 |
| 7 | 7.5 | 8 | 9 | 9 | 9.5 | 10 |
| 8 | 9 | 6 | 7 | 10 | 8 | 10 |
| 9 | 8 | 8 | 7 | 8 | 8 | 9 |
| 10 | 10 | 10 | 8 | 11.5 | 10 | 8 |
| 11 | 9 | 6 | 7 | 9 | 8 | 8 |
| 12 | 9 | 9 | 10 | 9 | 11 | 10 |
| 13 | 8 | 9 | 7 | 8 | 9 | 5.5 |
| 14 | 8 | 6.5 | 8 | 9 | 6.5 | 8 |
| *Mean* | 8.071429 | 7.678571 | 8.178571 | 8.857143 | 8.642857 | 8.964286 |
| *Max* | 10 | 10 | 10 | 11.5 | 11 | 11 |
| *Min* | 6 | 6 | 5 | 7 | 6.5 | 5.5 |
| *SD* | 1.08941 | 1.295067 | 1.488509 | 1.150728 | 1.231456 | 1.680937 |

*A, Aquarium; P, Pond; T, Treatment*

**Appendix Table 10:** Alkalinity level in 2013

| *Week No.* | *A-T*$_1$ | *A-T*$_2$ | *A-T*$_3$ | *P-T*$_1$ | *P-T*$_2$ | *P-T*$_3$ |
|---|---|---|---|---|---|---|
| 1 | 135 | 165 | 155 | 50 | 65 | 70 |
| 2 | 145 | 130 | 170 | 55 | 65 | 70 |
| 3 | 90 | 85 | 120 | 75 | 80 | 90 |
| 4 | 95 | 90 | 80 | 65 | 95 | 110 |
| 5 | 125 | 85 | 95 | 135 | 105 | 145 |
| 6 | 135 | 105 | 145 | 105 | 95 | 135 |
| 7 | 105 | 95 | 135 | 60 | 65 | 80 |
| 8 | 60 | 65 | 80 | 145 | 130 | 165 |
| 9 | 110 | 125 | 130 | 90 | 85 | 120 |
| 10 | 115 | 120 | 105 | 95 | 90 | 80 |
| 11 | 45 | 65 | 70 | 125 | 85 | 95 |
| 12 | 55 | 65 | 70 | 135 | 105 | 145 |
| 13 | 75 | 80 | 90 | 105 | 95 | 135 |
| 14 | 65 | 95 | 110 | 60 | 65 | 80 |
| *Mean* | 96.78571 | 97.85714 | 111.0714 | 92.85714 | 87.5 | 108.5714 |
| *Max* | 145 | 165 | 170 | 145 | 130 | 165 |
| *Min* | 45 | 65 | 70 | 50 | 65 | 70 |
| *SD* | 32.73478 | 28.804 | 32.35492 | 32.97518 | 18.98886 | 32.01133 |

*A, Aquarium; P, Pond; T, Treatment*

**Appendix Table 11:** Alkalinity level in 2014

| *Week No.* | *A-T*$_1$ | *A-T*$_2$ | *A-T*$_3$ | *P-T*$_1$ | *P-T*$_2$ | *P-T*$_3$ |
|---|---|---|---|---|---|---|
| 1 | 145 | 150 | 175 | 130 | 125 | 115 |
| 2 | 125 | 135 | 145 | 130 | 135 | 155 |
| 3 | 125 | 145 | 150 | 90 | 120 | 110 |
| 4 | 80 | 115 | 105 | 115 | 120 | 140 |
| 5 | 150 | 105 | 90 | 145 | 165 | 125 |
| 6 | 145 | 165 | 125 | 80 | 90 | 95 |
| 7 | 80 | 90 | 95 | 120 | 155 | 160 |
| 8 | 120 | 155 | 160 | 125 | 135 | 175 |
| 9 | 130 | 140 | 145 | 125 | 145 | 150 |
| 10 | 80 | 85 | 65 | 80 | 115 | 105 |
| 11 | 130 | 125 | 115 | 150 | 105 | 90 |
| 12 | 130 | 135 | 155 | 145 | 165 | 125 |
| 13 | 90 | 120 | 110 | 80 | 90 | 95 |
| 14 | 115 | 120 | 140 | 120 | 155 | 160 |
| *Mean* | 117.5 | 127.5 | 126.7857 | 116.7857 | 130 | 128.5714 |
| *Max* | 150 | 165 | 175 | 150 | 165 | 175 |
| *Min* | 80 | 85 | 65 | 80 | 90 | 90 |
| *SD* | 25.01922 | 23.59514 | 31.23159 | 24.77647 | 25.19157 | 28.10889 |

*A, Aquarium; P, Pond; T, Treatment*

**Appendix Table 12:** Ammonia-Nitrogen level in 2013 and 2014

| *Week No.* | *2013* | | | *2014* | | |
|---|---|---|---|---|---|---|
| | *A-$T_1$* | *A-$T_2$* | *A-$T_3$* | *A-$T_1$* | *A-$T_2$* | *A-$T_3$* |
| 1 | 0.0016 | 0.0017 | 0.0024 | 0.0016 | 0.002 | 0.0043 |
| 2 | 0.0011 | 0.0018 | 0.0022 | 0.0023 | 0.0017 | 0.0023 |
| 3 | 0.0014 | 0.0016 | 0.0019 | 0.0018 | 0.0022 | 0.0016 |
| 4 | 0.004 | 0.0017 | 0.0032 | 0.004 | 0.0056 | 0.0024 |
| 5 | 0.0014 | 0.003 | 0.0021 | 0.0045 | 0.0055 | 0.0016 |
| 6 | 0.0027 | 0.0021 | 0.0022 | 0.0018 | 0.0021 | 0.0016 |
| 7 | 0.0017 | 0.0016 | 0.0019 | 0.0016 | 0.0044 | 0.004 |
| 8 | 0.0017 | 0.0018 | 0.002 | 0.004 | 0.0056 | 0.003 |
| 9 | 0.0016 | 0.0045 | 0.0026 | 0.003 | 0.0032 | 0.0017 |
| 10 | 0.0027 | 0.0034 | 0.003 | 0.0016 | 0.0017 | 0.0023 |
| 11 | 0.0033 | 0.0018 | 0.002 | 0.0018 | 0.0018 | 0.0023 |
| 12 | 0.0016 | 0.0019 | 0.0021 | 0.003 | 0.0016 | 0.0061 |
| 13 | 0.0017 | 0.004 | 0.0036 | 0.0028 | 0.0018 | 0.0036 |
| 14 | 0.0018 | 0.003 | 0.0025 | 0.0017 | 0.004 | 0.003 |
| *Mean* | 0.002021 | 0.002421 | 0.002407 | 0.002536 | 0.003086 | 0.002843 |
| *Max* | 0.004 | 0.0045 | 0.0036 | 0.0045 | 0.0056 | 0.0061 |
| *Min* | 0.0011 | 0.0016 | 0.0019 | 0.0016 | 0.0016 | 0.0016 |
| *SD* | 0.000831 | 0.000975 | 0.000524 | 0.001025 | 0.001601 | 0.001292 |

*A, Aquarium; T, Treatment*

**Appendix Table 13:** Chlorine level in 2013 and 2014

| *Week No.* | *2013* | | | *2014* | | |
|---|---|---|---|---|---|---|
| | *A-$T_1$* | *A-$T_2$* | *A-$T_3$* | *A-$T_1$* | *A-$T_2$* | *A-$T_3$* |
| 1 | 0.008 | 0.01 | 0.038 | 0.01 | 0.066 | 0.01 |
| 2 | 0.009 | 0.008 | 0.056 | 0.01 | 0.065 | 0.063 |
| 3 | 0.018 | 0.009 | 0.01 | 0.008 | 0.01 | 0.008 |
| 4 | 0.045 | 0.045 | 0.032 | 0.074 | 0.008 | 0.01 |
| 5 | 0.033 | 0.067 | 0.031 | 0.036 | 0.01 | 0.009 |
| 6 | 0.009 | 0.008 | 0.056 | 0.008 | 0.01 | 0.008 |
| 7 | 0.018 | 0.009 | 0.01 | 0.074 | 0.008 | 0.009 |
| 8 | 0.045 | 0.045 | 0.032 | 0.036 | 0.01 | 0.018 |
| 9 | 0.033 | 0.067 | 0.031 | 0.031 | 0.036 | 0.045 |
| 10 | 0.01 | 0.038 | 0.01 | 0.066 | 0.01 | 0.01 |
| 11 | 0.007 | 0.056 | 0.01 | 0.065 | 0.063 | 0.032 |
| 12 | 0.009 | 0.01 | 0.008 | 0.01 | 0.008 | 0.031 |
| 13 | 0.038 | 0.01 | 0.066 | 0.01 | 0.036 | 0.045 |
| 14 | 0.056 | 0.01 | 0.065 | 0.063 | 0.01 | 0.01 |
| *Mean* | 0.024143 | 0.028 | 0.0325 | 0.035786 | 0.025 | 0.022 |
| *Max* | 0.056 | 0.067 | 0.066 | 0.074 | 0.066 | 0.063 |
| *Min* | 0.007 | 0.008 | 0.008 | 0.008 | 0.008 | 0.008 |
| *SD* | 0.016988 | 0.023732 | 0.021382 | 0.027251 | 0.023508 | 0.018064 |

*A, Aquarium; T, Treatment*

# Appendix 2: Benefit-cost related data

**Appendix Table 14:** Benefit cost ratio in aquaria and ponds in 2013 and 2014

| *Treatment* | *Replication* | *AG2012* | *AG2013* | *AD2012* | *AD2013* | *PG2012* | *PG2013* | *PD2012* | *PD2013* |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1.84 | 8.125 | -0.104 | 1.25 | 16.76667 | 17.83333 | 4.733333 | 4.8 |
| 1 | 2 | 0 | 6.225 | 0.024 | 1.7 | 12.26667 | 12.83333 | 5.6 | 5.8 |
| 1 | 3 | 1.592 | 6.8 | -0.344 | 1.95 | 17.53333 | 17.4 | 3.266667 | 4.066667 |
| 2 | 1 | -1 | 0 | -1 | -1 | -1 | 1.464865 | -1 | 0.491892 |
| 2 | 2 | -1 | -1 | -1 | -0.47059 | -1 | 2.308108 | -1 | 1.52973 |
| 2 | 3 | -1 | -0.5 | -1 | 0 | -1 | 1.854054 | -1 | 0.816216 |
| 3 | 1 | 1.851852 | 7.04 | 0.288889 | 2.12 | 11.3 | 10.4 | 3.56 | 3.26 |
| 3 | 2 | 0.733333 | 5.18 | -0.02222 | 1.68 | 9.83 | 10.61 | 3.14 | 3.92 |
| 3 | 3 | 1.237037 | 5.48 | 0.274074 | 1.52 | 10.58 | 8.6 | 1.58 | 3.02 |

*A, Aquaria; D, Dwarf gourami, G, Giant gourami; P, Ponds*