

Effects of different levels of temperature on some phenotypes of stripped dwarf catfish (*Mystus vittatus*)



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**Effects of different levels of temperature on some phenotypes of
stripped dwarf catfish (*Mystus vittatus*)**

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Declaration

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

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DEDICATION

**This thesis is dedicated to my Beloved PARENTS. Who sacrificed for me, inspired
and blessed me in every ways.**

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Abstract

In an era of rapid climate change, there is crying need to understand how organisms will cope with faster and less predictable variation in environmental conditions. Several studies show that temperature is one of the most important climate change variables for all aquatic organisms especially for fish because it can directly affect different life history traits. Therefore, the present study was carried out under laboratory condition to explore the expression of some phenotypic traits by manipulating water temperatures using a popular freshwater fish species, the striped dwarf catfish (*Mystus vittatus*). The study assigned almost same sized juvenile fish haphazardly to one of three experimental temperatures (i.e. ambient: 26-28°C, low: 20-22°C, high: 30-32°C) and each of these temperature treatments was further divided into two replications. Each replication contained 15 good looking and healthy juveniles up to one month of the experimental period. Thus, the experimental design was able to explore the effects of temperature manipulation on the expression of some selected phenotypic traits (e.g. body length and size, colour patterns, egg and sperm numbers etc.) After one month of experiment, the study revealed that temperature manipulations had significant effects on some of the phenotypic traits in *M. vittatus*. Fish reared in low temperature showed significantly higher body weight than the hot and ambient treatment group. The study also revealed that high treatment group had significantly brighter/darker coloured fish than the low and ambient treatment group, while the ambient treatment group had significantly higher number of normal/natural coloured fish than high and low treatment group. Moreover, the study showed that the low treatment group fish possessed significantly higher number of eggs as well as sperm than the high and ambient treatment group. However, no significant effect of temperature manipulation was found on standard length, tail length, head length and body area. These results further underscore the sensitivity of some phenotypic traits of *M. vittatus* under different level of temperatures and highlight the important role of environmental temperature.

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Chapter- 1

1.0 Introduction:

1.1 Background of the study:

Ecological and environmental conditions make the evolution of life history traits in many species (Metcalf and Monaghan, 2001). Animals' growth (Barlow *et al.*, 1995; Burel *et al.*, 1996), survival (McLeese, 1956; Kasper and Tamplin, 1993), physiology (Jackson, Palmer and Meadow, 1974; Bartness and Clein, 1994) and reproduction (Ammon, 1951; McCabe and O'Brien, 1983) etc. can be influenced by different ecological conditions. These life history traits are different for different organisms and can also vary in different environmental conditions (Snell-Rood *et al.*, 2015). It has been observed that the manipulation of environmental parameters like temperature (Kasper and Tamplin, 1993), salinity (Castillo-Vargasmachuca, *et al.*, 2013), photo period (Barlow *et al.*, 1995), oxygen level (Sreelatha, 2003), nutrient level and food availability (Snell-Rood *et al.*, 2015) etc. can alter the expression of various phenotypes or other biological traits in many animals.

Among various environmental factors, temperature is considered one of the most crucial factors which can greatly influence several phenotypic traits in many aquatic organism especially for fish. Fish are poikilothermic that means they are unable to internally regulate their body temperature. They are influenced by the temperature around them. For example, if the water is warm, fish metabolism accelerates, feeding and respiration increases but if the water is cold, fish become lethargic and tend to be inactive (Laudian *et al.*, 1981). Temperature is also essential because it can influence the water chemistry. The rate of chemical reactions generally increases at higher temperature, which affects the biological activity. In warm water, the fish eat more and their growth and reproduction rate is higher (Chatterjee *et al.*, 2004). On the other hand, the cold water fish require low energy, less feed and their growth rate is slow (Leris *et al.*, 2011).

Fish can innure or adapt themselves with the changing environment through phenotypic plasticity that occurs when individuals of a given genotype adjust their phenotype according to the

conditions they experience (Schlichting *et al.*, 1996; West-Eberhard, 2003). Many studies have reported a role for plasticity in shaping phenotypic responses to contemporary disturbances such as temperature change (Charmantier *et al.*, 2008; Gienapp *et al.*, 2008; Hendry *et al.*, 2008; Teplitsky *et al.*, 2008). Plasticity in stress tolerance usually the exposure of an organism to particular conditions that changes response to a challenge (Angilletta, 2009; Kassahn *et al.*, 2009). For example, prolonged warm acclimation enhances high-temperature tolerance in killifish (*Fundulus heteroclitus*), but repeated heat shocks do not (Healy *et al.*, 2012). In Stripped dwarf catfish (*M. vittatus*), developmental plasticity affects acclimation to temperature substantially later in life (Scott and Johnston, 2012).

An illimitable number of literatures have drawn attention to anticipate the phenotypic consequences of temperature change in fishes (Laudian *et al.*, 1980; Laudien *et al.*, 1981; Axelrod *et al.*, 1985; Pörtner, 2001; Francis *et al.*, 1993; Fry, 1971; Montalvo *et al.*, 2012a and b; Chatterjee *et al.*, 2004; Fielder *et al.*, 2005; Ramler *et al.*, 2014; Leris *et al.*, 2011; Gibson, 1954; Arai *et al.*, 1963). Ramler *et al.* (2014) carried out an experiment to observe the effects of temperature on body form and developmental canalization in the three spine stickleback under three different temperature regimes (13, 17 and 21°C). The temperature induced differences between 13 and 17°C (comprising relative head and eye size) deviated considerably from those between 17 and 21°C. For both size and shape phenotypic variance was significantly smaller at 17°C than at 13 and 21°C. Leris *et al.* (2011) conducted a study to investigate the temperature effect on swimming ability of four populations of zebrafish, reared in different water temperatures (22, 25, 28 and 31°C). They mentioned fish that were reared in 22°C showed significant lower performance than the ones reared in 31°C.

Although many studies were carried out to explore how temperature variation can affect different life-history traits in various species, very limited studies exposed the impact of different temperatures on various phenotypes of commercially important freshwater fish species like the striped dwarf catfish (*Mystus vittatus*). The Stripped dwarf catfish (*M. vittatus*) is native to fresh and brackish waters of Bangladesh, the Punjab, Sind Ceylon, India, Assam, Myanmar, Siam, Nepal, Sri Lanka and Thailand (Bhuiyan, 1964; Talwar and Jhingran, 1991). They are mostly available in freshwater bodies, flooded canals, beels (especially Chalan beel), paddy and jute

fields, streams, haors, oxbow lakes, tidal zone and rivers in swarms during rainy season (Bhuiyan, 1964; Galib *et al.*, 2009; Shafi and Quddus, 2001; Talwar and Jhingran, 1991). Their colour varies with age, generally delicate gray-silvery to shining golden, with about 5 pale blue or dark brown to deep black longitudinal on side (BdFISH, 2010). Their maximum length could be 11.7 cm (Rahman, 1989 and 2005). They usually breed during summer monsoon (Bhuiyan, 1964; Shafi and Quddus, 2001). For food and feeding they take algae (18%), higher plants (27%), protozoa (13%), crustaceans (24%) and insects (11%) (Bhuiyan, 1964). According to Shafi and Quddus (2001) they are bottom feeder and prefer algae, aquatic plants, protozoans, and other aquatic insect larvae. *M. vittatus* has a great importance as food. The fish is very popular in Bangladesh and other south Asian countries. People prefer *M. vittatus* for its good taste, high nutrient value and availability throughout the year. This species is also enriched with many minor and trace elements, such as sodium, potassium, calcium, iron, iodine, zinc, magnesium and phosphorus (Hossain, 1999; Rao *et al.*, 1999). According to Gupta (2014) this fish has a good market demand and recently become popular as an ornamental fish. So, it can also be used as aquarium fish species for its beautiful colour pattern and now a days it has been started to culture for commercial purposes.

Some studies already have been carried out about the distribution and habitat of *M. vittatus* (Bhuiyan, 1964; Galib *et al.*, 2009; Gupta, 2014; Shafi and Quddus, 2001; Talwar and Jhingran, 1991). Some other researchers investigated about the feeding and breeding biology of them (Azadi *et al.*, 1987; Bhatt, 1971; Bhuiyan, 1964; Gupta 2014; Islam *et al.*, 2011; Shafi and Quddus, 2001; Welliang and Amarasinghe, 2007). Researchers also conducted some experiments on morphology of *M. Vittatus* (Rahman, 1989 and 2005; Shafi and Quddus, 2001; Talwar and Jhingran, 1991). Especially some studies have been carried out about the maximum length of the fish (Bhuiyan, 1964; Hussain, 1999; Rahman, 1989 and 2005; Shafi and Quddus, 2001). According to Shafi and Quddus (2001) maximum length of *M. vittatus* is 20.2 cm. Although the above mentioned studies have been conducted, only a few number of studies have been taken to measure the effects of temperature on the expression of selected traits in this important species (e.g. Karayucel *et al.*, 2008; Breckels *et al.*, 2013). Hence, the present study has been designed to explore the expression of some phenotypic traits (i.e. body length and area, colour patterns, egg and sperm number etc.) in a very important freshwater fish, the striped dwarf catfish (*M. vittatus*) under different temperatures.

1.2 Objectives of the study:

The major aims of this study were:

- ❖ To know the effect of various temperatures on survival and growth of *M. vittatus*;
- ❖ To explore the change of different colour patterns of experimental fish with temperature manipulation;
- ❖ To investigate the influence of temperature modulation on the quantity of egg and sperm in the experimental fish.



Chapter-2

2.0 Literature Review:

2.1 General Introduction:

In this world, man is the only animal who can take benefits of knowledge which has been accumulated through the centuries. So, literature review is a critical and evaluative account of what has been published on a selective research topic. Literature reviews are indispensable when planning a research study because it helps researchers and readers becoming familiar with the work. Tolerance limits of fishes to different temperatures are of ecological significance in assessing fish distribution and their impact on ecosystems. *M. vittatus* is a member of the Bagridae family from Siluriformes order and mainly a freshwater species, but is also found in brackish water. The present study has generated an interest to investigate to explore whether the variation in temperatures can influence the expression of some phenotypes in *M. vittatus*.

2.2 Effects of environmental factors and their importance:

2.2.1 Effects of environmental factors:

An individual in environment develops its phenotype and environment also assumes a greater role in evolution, and in fact, may produce more viable phenotypic variation (West-Eberhard, 2003 and 2005). Phenotypes are the outcome of complex interactions between genes and environment (Gilbert *et al.*, 2008). The influence of environment is more conspicuous during early stages of life (Lindstrom, 1999).

Among different abiotic factors, ambient temperature has recently shown to be a proximate factor significantly affecting the expression of precopulatory (Eraud *et al.*, 2007; Bajer *et al.*, 2012) and postcopulatory (Vasudeva *et al.*, 2014) traits with specific evidence show that variation in temperature during early developmental period can influence the expression of phenotype in different fish species (Jonsson, 2012; Scott and Jonsson, 2012; Steinbacher *et al.*, 2011). Acid exposure can affect the gill in various ways, such as by increasing the number and turnover of ion-

transporting cells (ionocytes), elevating mucus production and recruiting leukocytes (Balm and Pottinger, 1993; Fromm, 1980; Laurent and Perry, 1991; Leino and McCormick, 1984; Leino *et al.*, 1987; Bonga *et al.*, 1990). Breakdown of gill structure and suffocation caused by mucus accumulation may also occur in fish exposed to extreme acidic water (pH 2.0–3.5) (Packer and Dunson, 1972). Besides, dissolved oxygen can affect the aquatic organism greatly. As dissolved oxygen levels in water drop below 5.0 mg/l, aquatic life is put under oppress. Oxygen levels that remain below 1-2 mg/l for a few hours can result in large fish kills (Breitburg *et al.*, 1997). The intensity of the light in the rearing environment of marine fish larvae can be a significant factor in survival and growth. Nutritional status is also considered a key regulator restrictive the expression of condition dependent sexual traits (Nowicki *et al.*, 2002; Cotton *et al.*, 2004; Rahman *et al.*, 2015; Rahman *et al.*, 2014). Accordingly, studies have revealed that the expression of fitness-enhancing phenotypic traits in numerous species can influenced by the levels of dietary nutrients (Rahman *et al.*, 2014; Smith *et al.*, 2007; Ward *et al.*, 2011; Tigreros, 2013). Together, such changes reduce the competitive ability of organisms with subsequent negative effects on reproductive success and fitness (Lummaa and Clutton-Brock, 2002; Arendt, 2000).

2.2.2 Importance of environmental factors:

Environmental factors are those physical or chemical parts of the environment that allow or inhibit an organism to grow and reproduce, maintain their structures, and respond to their environments. Major environmental factors are temperature, salinity, pH, light, current and water turbulence, dissolved oxygen levels, nutrient, depth, etc. that have a great impact on organisms. In an environment there is an interaction between the environmental factors and organisms. The development (phenotypic or genotypic) of an individual depends on environment directly or indirectly that gradually causes evolution of an organism (West-Eberhard, 2003). Among environmental factors in aquatic habitats, water temperature is very important that has a great effect on aquatic ecosystem, as it affects the organisms, even the chemical and physical characteristics of water (Delince, 1992; Mohamed, 2005). Salinity is one of the most important factors in aquatic environment (Mohamed, 2005) and shows various effects on the vitality of different organisms (Chand *et al.*, 2015; Küçük, 2013; Lisboa *et al.*, 2015; Uka *et al.*, 2013). Another vital factor is pH in the environment as all aquatic organisms live in around pH 7. They can even die if it changes (Araoye, 2009). Other environmental factors like dissolved oxygen

(Araoye, 2009), light (Barlow *et al.*, 1995), dietary nutrients (Rahman *et al.*, 2014), etc. also can greatly affect the physiology and other life history traits of the organisms.

2.3 Importance of temperature to organisms:

2.3.1 Impact of temperature on aquatic organism:

Temperature is considered as the 'abiotic master factor' for fishes (Brett, 1971). Most climate change research to date has focused on effects of increasing temperatures (Doney *et al.*, 2009; Kroeker *et al.*, 2013). Surrounding water temperature influence metabolic processes, behaviour, migration, growth, sex ratio, reproduction, and survival of fish due to fluctuation of temperature. (Pörtner, 2001; Francis and Barlow, 1993; Fry, 1971). Such as, if the water is warm, fish metabolism accelerates, feeding and respiration increases but if the water is cold, fish become lethargic and tend to be inactive (Montalvo *et al.*, 2012a and 2012b). A certain species adversely affects fish health for beyond the optimum limits of temperature by increasing metabolic rate and subsequent oxygen demand (Chatterjee *et al.*, 2004). Fish growth rates increase with increasing temperature up to a certain level, after which they sharply decline (Fielder *et al.*, 2005).

2.3.2 Influence of temperature on fish traits expression:

Environmental factor affects an organism in its embryonic stage and it may continue throughout the whole life cycle more or less. Global warming will be more problematic for obligate freshwater organisms. For fish this problem is further compounded because of their poikilothermic nature whereby their basic physiology is directly dependent on the temperature of their environment (Kaushal *et al.*, 2010). Climate change, particularly the increased temperature predicted for the end of the century, has the potential to alter many life history traits, including juvenile survival (Zeh *et al.*, 2012). In the juvenile stages, most of the fish species show a rapid specific growth rate with increasing temperature at its peak growth, then falling rapidly as high temperatures become adverse (Brett, 1979). The physiological response of *Clarius batrachus* at different temperatures were observed. Fish were acclimated at 25°C for 21 days and were exposed at 10, 15, 20, 25, 30 and 35°C temperatures. Significantly ($p < 0.05$) higher mortality of fish was found in 10°C treatment compared to the other groups. Thiobarbituric acid reactive substance was significantly ($p < 0.05$) higher in fish exposed at 10 and 15°C compared to others after 12h and 7 days of exposure,

respectively. Lowering of temperatures adversely affected physiological process of magur (Singh *et al.*, 2013).

Temperature affects the ventilator behaviour of fish such as the amplitude of silver perch increases with increasing temperature (Patra *et al.*, 2009). In a tropical reef fish, reproductive performance decline with increasing temperatures, where 64% of fish reproduce in normal water temperature and 36% of fish reproduce if temperature increases 3°C (Donelson *et al.*, 2014).

2.4 Biology and life history of stripped dwarf catfish

2.4.1 Taxonomy

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Siluriformis

Family: Bagridae

Genus: *Mystus*

Species: *M. Vittatus*

Binomial name: *Mystus vittatus* (Bloch, 1794)



Figure 1: Some pictures of experimental species stripped dwarf catfish (*M. vittatus*).
[Source: <https://www.google.com>]

2.4.2 Distribution and habitat

M. vittatus (Bloch, 1794) is a freshwater fish species and generally known as Asian striped dwarf catfish but in locality it is known as Tengra. This species mainly occurs in inland water bodies of India, Bangladesh, Pakistan, Sri Lanka, Thailand, Nepal and Malaysia. In Bangladesh it is fresh water small indigenous species (SIS) and commonly occurring in inland water areas, also found in canals, beels (Chalan Beel), paddy and jute fields, streams, haors, and irrigation channels; usually inhabits marginal vegetation in lakes and swamps with muddy substrates (Bhuiyan, 1964; Galib *et al.*, 2009; Gupta, 2014; Shafi and Quddus, 2001). Even in the tidal zone both standing and flowering water bodies it has been found (Talwar and Jhingran, 1991).

2.4.3 Food and feeding behaviour

M. vittatus takes diversified food items like copepods, cladocerans, rotifers, ostracods, insects, oligochaetes, chlorophyceae, bacillariophyceae and debris that have been documented in its gut content. But it has been recorded that they are plankton feeder and prefer zooplankton. Copepods, insect larvae, rotifer and daphnids are main food items for this species (Bhatt, 1971). For food and feeding they take algae (18%), higher plants (27%), protozoa (13%), crustaceans (24%) and insects (11%) (Bhuiyan, 1964). According to Shafi and Quddus (2001), they are bottom feeder and food of this fish composed of 22% algae, 27% aquatic plants, 13% protozoans, 2% chironomas and other aquatic insect larvae, 16% small mollusc and 20% small fishes. Zoobenthos contribute 22% of *M. vittatus*'s diet with insect larvae as major component (Welliang and Amarasinghe, 2007).

2.4.4 Morphology

M. vittatus has an elongated body that is slightly compressed. It has 4 barbels and a weak dorsal spine. Head 23.3% of SL and 18.2% of TL. Height 16.3% of SL and 12.7% of TL. Eye 15% of HL (Rahman, 1989 and 2005; Shafi and Quddus, 2001; Talwar and Jhingran, 1991). It has a variety of body length. Maximum lengths is 10.2 cm (Bhuiyan, 1964); 9.5 cm (Hussain, 1999); 11.7 cm (Rahman, 1989 and 2005) and 20.2 cm (Shafi and Quddus, 2001). Lateral line is present and straight. It tolerates comparatively wide ranges of salinities. Females are comparatively larger in size than the males.

2.4.5 Colour patterns

Colour of *M. vittatus* varies with age and normally it is delicate gray-silvery to shining golden, with about 5 pale blue or dark brown to deep black longitudinal on side (BdFISH, 2010). Colours vary from strain to strain. A narrow dusky spot often present on the shoulder. The fins glass, with dark tips (BdFISH, 2010).

2.4.6 Reproductive biology

In *M. vittatus*, with the presence of genital papilla, male and female can be distinguished externally as it is present in males and absent in female fishes. In the breeding season this papilla becomes very noticeable (Bhatt, 1971). According to Bhatt, female dominance over male in *M. vittatus* population and later it has been supported by Rao and Sharma (1984). In this species male and female acquire their first maturity at 8.5 cm and 9 cm length (Rao and Sharma, 1984). The fecundity of *M. vittatus* is 2,515 to 9,789 (Azadi et al., 1987). *M. vittatus* has a positive correlation of fecundity with body weight, total length and ovary weight (Azadi et al., 1987 and Islam et al., 2011). Breeding season is the summer monsoon (Bhuiyan, 1964; Shafi and Quddus, 2001). But according to Rao and Sharma (1984) *M. vittatus* spawns between September and October and peak spawning occurs in October.

2.5 Previous research about the effects of temperature on fish phenotypes

2.5.1 Survival and Growth

❖ Neotropical catfish (*Lophiosilurus alexandri*)

According to D. P. da Costa et al. (2016) the appropriate temperature of juvenile *L. alexandri* for growth is between 27 and 28°C. A study was conducted to analyse the effects of different water temperatures on the growth, survival and blood physiological parameters of *Lophiosilurus alexandri*. Juveniles measuring 12.6 cm and weighing 30.6 g were subjected to four water temperature regimes: 23, 26, 29 and 32°C with four replicates. Survival was 100% at all water temperatures, and the best temperature for growth was estimated to be 27.8°C using a quadratic regression model.

❖ Yellowtail kingfish (*Seriola lalandi*)

To assist in determining the most appropriate water quality conditions for this species in RAS, the effect of water temperature (21, 23.5, 25, 26.5 and 29⁰ C) and PH (6.58, 7.16, 7.85) was tested in two separate experiments by W. Abbink *et al.* (2011). Growth performance, feed conversion and metabolic parameters were assessed in juvenile yellowtail kingfish grown in pilot scale RAS. Growth was optimized at a water temperature of 26.5⁰ C, in combination with maximum food intake and FCR. Increasing temperature from 21-26.5⁰ C resulted in a 54% increase in the fish's final weight after 30 days.

❖ Nile tilapia (*Oreochromis niloticus*)

A study was conducted to investigate the effect of high water temperature on survival and growth performance of Nile tilapia by Pandit and Nakamura, 2010. Two age groups of female Nile tilapia (9 and 50 days after hatching) were reared at 27, 32, 35 and 37⁰ C water temperatures for 45-60 days. At the end of treatment the mean survival, total weight, total length and FCR of both age groups of fishes were significantly reduced at 35 and 37⁰ C compared to 27 and 32⁰ C ($p < 0.05$). It was concluded that higher temperature ($> 32^{\circ}$ C) results in slow growth, reduce feeding efficiency and increase mortality (Pandit and Nakamura, 2010).

2.5.2 Behaviour and Colour pattern

❖ Zebrafish (*Danio rerio*)

Adverse environmental condition like acute stress may affect metabolism and nitrogen excretion in animals as part of the adaptive response. The acute effects of different temperatures and salinities on routine metabolism, spontaneous activity and excretion of ammonia and urea are studied in fresh water fish: zebrafish at 27⁰C temperature. In zebrafish four different salinities (0, 10, 20 and 25 ppt) were tested by Uliano *et al.* (2010). Acute changes in temperature and salinity severely affected any parameter were tested both alone and in combination. A reduction of nitrogen excretion at 20 °C and 25 ppt was the main effect of the observations as under these extreme conditions a significant structural disruption of gill was observed.

❖ Frillfin goby (*Bathygobius soporator*)

Fanta (1997) conducted an experiment to determine the behaviour and colour pattern of frillfin goby under different salinities (8.5, 17.0, 25.5 and 34.0 ppt) and temperatures (18°C and 28°C). Swimming was rare and decreased (day time) with increasing temperature and low salinity.

There was a tendency of the presence of some dark fish during the morning and some light ones in the afternoon, showing a higher variability of colours at low temperature and extreme salinities (Fanta, 1997).

2.5.3 Gamete and Reproduction

❖ Medaka (*Oryzias latipes*)

To determine quantitative impact of reproductive endpoints, groups of medaka (two breeding pairs per group) were subjected to varying temperature and photoperiod regimes by Koger *et al.* (1999). Embryo production ceased after photoperiod reduction from 16 L: 8D to 8L: 16D (at 25°C). A severe decline in production was observed after a temperature decrease of 10°C (25 to 15°C [L: D]). Under both reduced photoperiod and reduced temperature regimes, mature spermatozoa were observed. Temperature change had no effect on offspring viability but caused an increase in spawning interval.

❖ Brown trout (*Salmo trutta*)

Fenkes *et al.* (2017) revealed that thermal impacts on gamete quality have been investigated on brown trout (*Salmo trutta*) and also mentioned that early in the season, warm acclimation of males reduced their fertilization probability (lower sperm velocity) when compared with cold-acclimated males, especially when the activation water temperature was also increased beyond the thermal optimum (resulting in a lower proportion of motile sperm with lower velocity).

2.6 About the present study

From the above vast information collected from a huge number of literatures (e.g. books, journals, webs, etc.), it can easily state that environmental factors have a great impact on aquatic animals especially fish. Among them temperature is a very important ecological factor. Although a great number of studies already has been carried out, very few of them have revealed a conspicuous picture of temperature impact on a commercially important fish species like *M. vittatus*, and therefore the present study has generated an interest to me to explore whether the variation in temperatures can influence the expression of some phenotypic traits, such as growth performance, colour patterns, sperm and egg number in *M. vittatus*.

Chapter-3

3.0 Materials and Methods:

3.1 Fish collection and acclimatization:

At first 200 juvenile striped dwarf catfish (*M. vittatus*) were collected from the local dealers of Khulna city. The fish were transported in oxygenated container to the Wet Fish Laboratory of FMRT Discipline where the experiment was conducted. They were then transferred in a large round water tank and conditioned for two days before starting the experiment. Finally, they were stocked in their experimental glass aquariums (50cm×29cm×30cm) for this experiment.

3.2 Water quality management:

The aquariums were cleaned up prior to stocking of fish and fitted with continuous aeration that was covered with net to avoid fish escaping. About half of the water was removed by siphoning thrice a week to clean the faces and uneaten food. Adhered dirt inside the aquarium walls was cleaned also thrice a week. Continuous aeration was ensured for rearing throughout the experimental periods. During the experimental period, temperature of water was monitored and maintained daily. Temperature reading was taken with a Celsius thermometer as well as with a digital thermostat.

3.3 Feeding:

The fish were fed insects egg, larvae and flash of small shrimp including a small amount of commercial supplementary fish feed in every day (except Friday). Exactly optimum and same amount of feed was given to all fish to avoid the feeding effect.

3.4 Experimental procedure:

3.4.1 Experimental design

There were three temperature treatment groups [e.g. ambient (26-28°C), high (30-32°C) and low (20-22°C) temperature] and each treatment had two replications where each replication possessed 15 almost same sized *M. vittatus* in individual tank. Thus six (6) tanks of this experiment contained almost same sized 90 sorted healthy and good looking *M. vittatus* in total (i.e. each treatment had 30 fishes). Temperatures were maintained by different thermostats throughout the experiment. Every aquarium was adorned with continuous aeration and covered with net to avoid fish escaping.

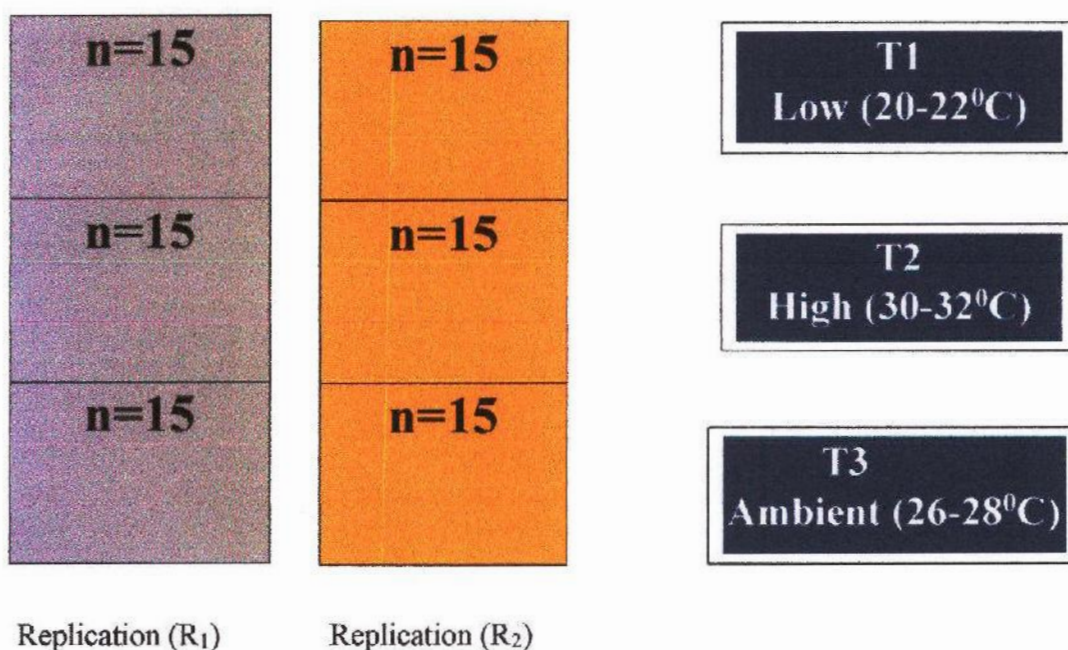


Figure 2: The overall experimental design of the study.

3.4.2 Determination of survival and growth

Fish survival was monitored and recorded regularly for each tank. At first, fish was placed on a graph paper and the photograph was taken by a digital camera (Canon DS126621). All the raw images were imported into *ImageJ* (v1.50) software for the measurements of selected phenotypic traits. Standard length (the distance in cm from the fish snout to the tip of his caudal peduncle),

tail length (the distance in cm from the fish caudal peduncle to the end of the tail), body area (the area [in cm^2] of the body excluding all fins and tail) were measured carefully.



Figure 3: Measurement of standard length (SL).

3.4.3 Observation of colour pattern

The previously described raw images were also carefully observed to detect the overall fish colour (i.e. how many fish were dark / bright, while others are normal/natural colour after the experimental period).

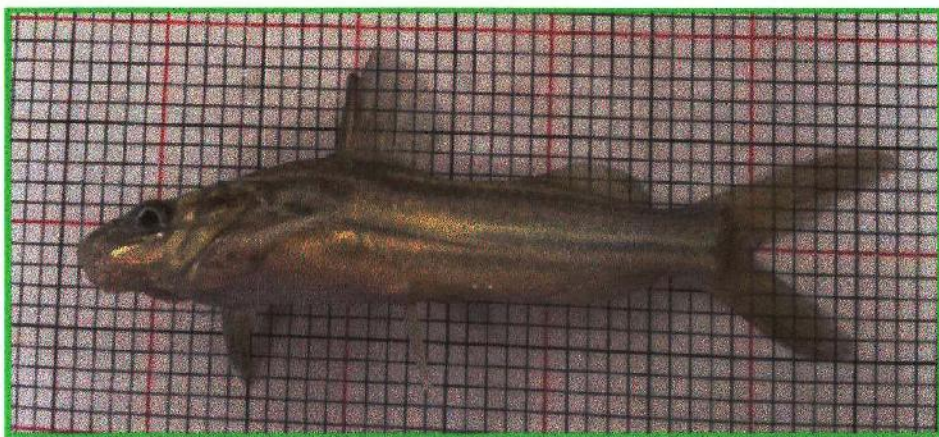


Figure 4: Normal coloured *M. vittatus*.



Figure 5: Dark coloured *M. vittatus*.

3.4.4 Egg and sperm number

After taking photo, each fish was anesthetized dipping in ice and then ovary of was collected from the individual female by dissecting. The total weight of the ovary was taken by electronic balance. A small portion of the ovary was taken from total ovary and weighed. Sperm was collected following the same procedure. The total weight of the sperm was taken and a small portion was taken from total sperm pool and weighed by electronic balance. Finally, egg and sperms were counted using an *improved Neubauer haemocytometer under 40 $\times$ magnification* (LABOMED, Lx 400 microscope).

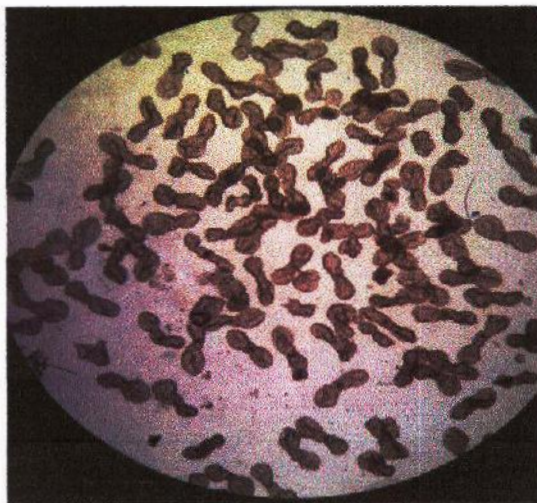


Figure 6: a) Sperm under microscope and b) Egg of experimental fish

3.5 Statistical analyses:

All analyses were conducted using 'RStudio' version 1.0.143(R Core Team 2017). The descriptive statistics (means, SD, SEs, etc.) were calculated using the 'psych' package (Revelle 2017), normality and homogeneity were tested with the 'car' package (Fox and Weisberg 2011). Appropriate transformation was applied to yield normal distributions for all interested traits.

The anova model was applied using 'car' package (Fox and Weisberg 2011) where target trait was included as a response variable and treatment was considered as fixed factor. When the target trait was not normally distributed, I used the Kruskal-Wallis test. While the trait was also not homogeneous, I used the Welch's Heteroscedastic F test.

Science egg and sperm numbers are count data, I used the generalized linear model (glm) with a 'quasipoisson' family option to control the overdispersion using the 'lme4' (Bates *et al.*, 2015). In the model, egg or sperm number was fixed as a response variable, while treatment was included as the fixed factor.

To compare the variation in colour patterns (i.e. categorical data), the Pearson's chi-squared test with Yates' continuity correction was performed using 'gmodels' (Warnes, *et al.*, 2015) and

‘rcompanion’ packages (Mangiafico, 2017). The mosaic plot was made to depict the status of different fish colour throughout the experiment between different treatment groups using ‘vcdExtra’ package (Friendly, 2016). Finally all graphs were made using ‘ggplot2’ package (Wickham 2009).

Chapter-4

4. Results

4.1 Effects on growth parameters:

Initially, there was no significant variation in body weight among the different treatment groups ($F_{2,87}=0.24$ and $P=0.09$). To explore the variation in growth between different treatment groups, body weight (BW), standard length (SL), tail length (TL), head length (HL) and body area (BA) were measured after rearing them up to one month at different temperatures. Mortality records showed only one fish in high and another one in ambient temperature were died during the entire experimental period.

4.1.1 Body weight:

After one month of experiment, I found significant effect of temperature on total body weight ($F_{2,84}=8.01$ and $P<0.001$). The subsequent post-hoc (TukeyHSD) test showed that the low treatment group fish had significantly higher body weight than the high treatment group ($P<0.001$), while the ambient treatment group also had significantly higher body weight than the high treatment group ($P<0.05$) and (figure 7). The analysis also revealed no significant variation in body weight between low and ambient treatment group ($P=0.42$)

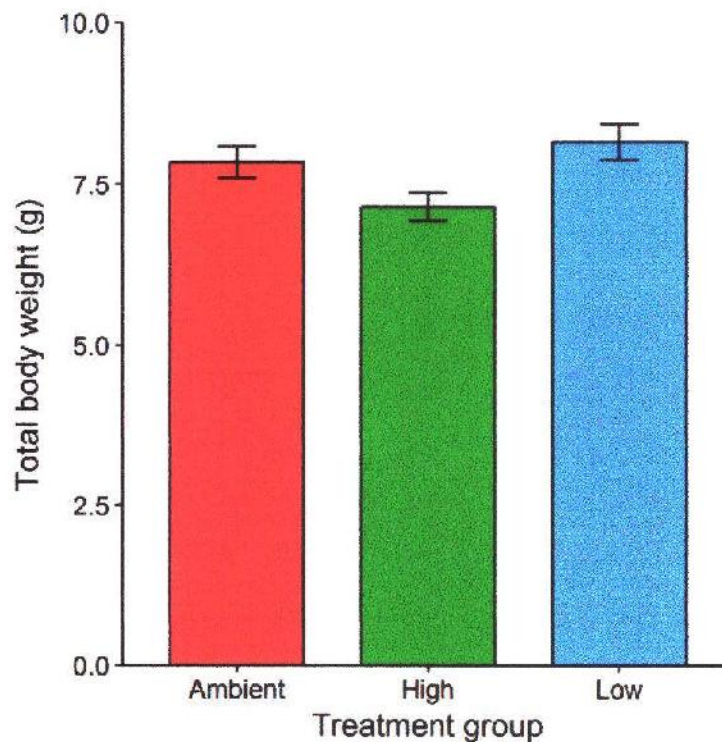


Figure 7: Variation in total body weight (mean $\pm$ SE) among different treatment groups.

4.1.2 Standard length:

The results revealed no significant variation in standard length among the different treatment groups ($F_{2,85}=2.01$, $P=0.14$).

4.1.3 Tail length:

Like standard length, the study also found no significant difference among the treatment groups ($F_{2,85}=1.01$, $P=0.37$).

4.1.4 Head length:

The experiment also disclosed that there was no significant variation in head length among the different treatment groups ($F_{2,85}=1.13$, $P=0.88$).

4.1.5 Body area:

The body area data also showed no significant variation among the different treatment groups like some other phenotypic traits ($F_{2,85}=1.95$, $P=0.15$).

4.2 Colour patterns:

The experimental fish had four strips in each side of their body and there were two different colours of tengra found during the experimental period, e.g. highly bright/dark and normal/natural colour.

4.2.1 Fish colour:

Dark colour: The Pearson's Chi-squared test with Yates' continuity correction revealed that high treatment group had significantly more bright/dark coloured fish than ambient ($P<0.05$), while no significant variation was found between high and low ($P=0.07$), and between ambient and low treatment group ($P=0.57$, and figure 8).

Normal colour: The Pearson's Chi-squared test also discerned that ambient treatment group had significantly higher number of normal/natural coloured fish than high ($P<0.05$), while no significant variation was found with low treatment group ($P=0.57$, and figure 8). There was also no significant variation was observed between high and low treatment group ($P=0.07$).

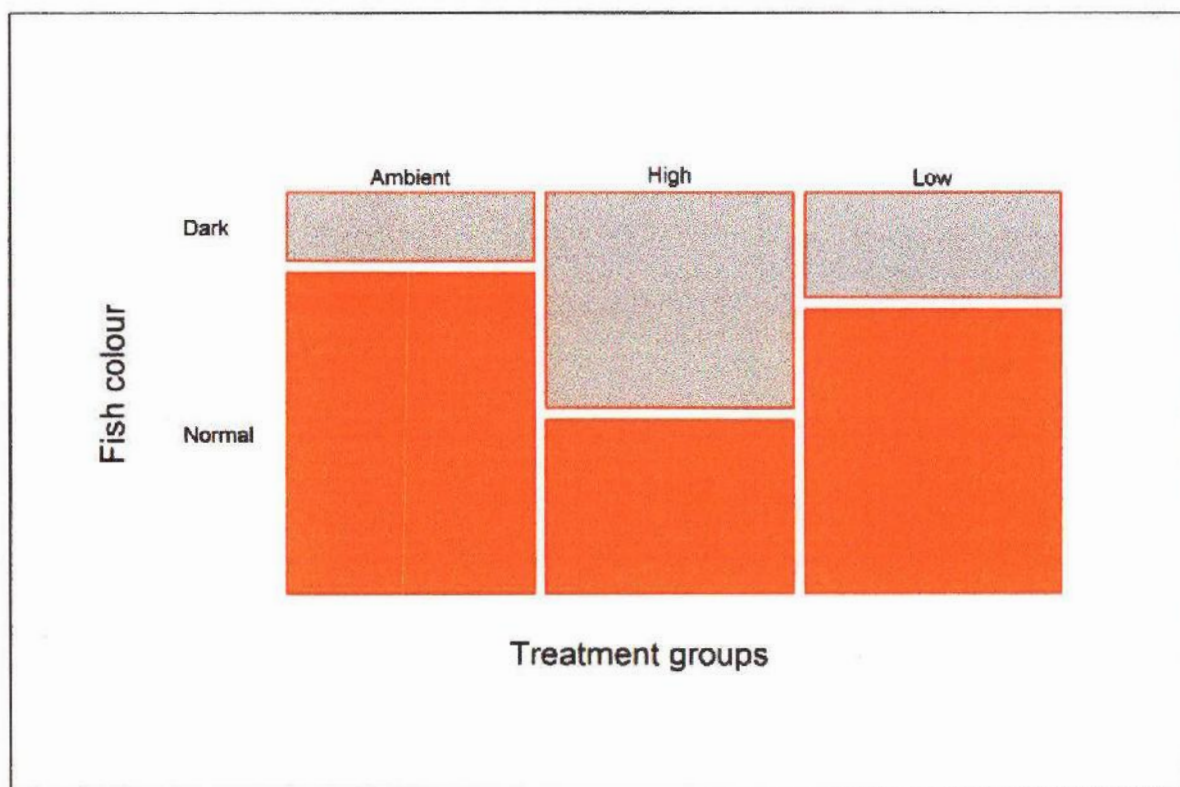


Figure 08: Mosaic plot showing body colour variation among different treatment groups after the experimental period.

4.3 Variation in reproductive traits:

4.3.1 Egg number

The egg number counting of different treatments revealed a significant variation among them ($F_{2,77}=9.85$ and $P<0.001$). The subsequent post-hoc (TukeyHSD) test showed that the low treatment group fish possessed significantly higher number of eggs than the high treatment group ($P<0.05$), while the ambient treatment group also had significantly higher number of eggs than the high treatment group ($P<0.01$). There was no significant variation found in egg numbers between ambient and low treatment group ($P= 0.61$ and figure 9)

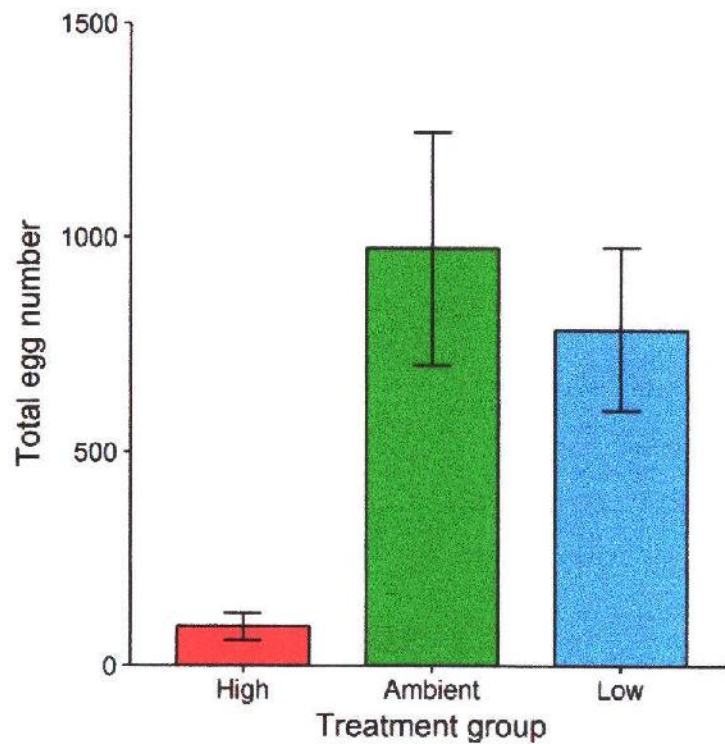


Figure 9: Variation in egg numbers (mean $\pm$ SE) among different treatment groups.

4.3.2 Sperm number:

The experiment revealed a significant variation in sperm counting among different treatment groups ($F_{2,77}=7.75$ and $P<0.001$). The subsequent post-hoc (TukeyHSD) test showed that the low treatment group fish had significantly higher number of sperm than the high ($P<0.01$) and ambient ($P<0.05$) treatment group, while no significant variation was found between ambient and high treatment group ($P= 0.65$ and figure 10)

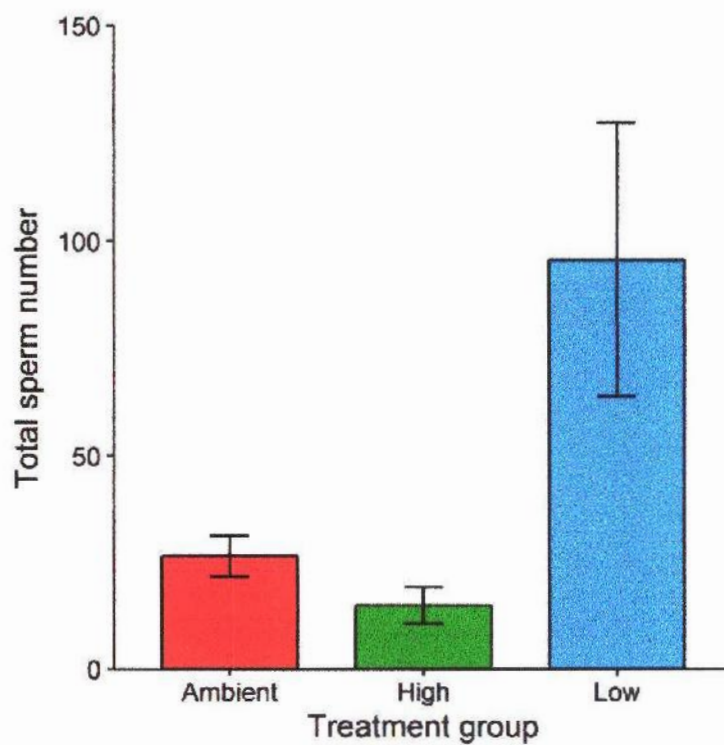


Figure 10: Variation in sperm numbers (mean $\pm$ SE) among different treatment groups.



Chapter-5

5.0 Discussion

The overall study has revealed that temperature manipulations have a significant effect on the expression of some phenotypic traits (e.g. body weight, colour pattern, egg and sperm number) in the experimental stripped dwarf catfish (see figures 7, 8, 9 and 10). On the other hand, it has also shown that temperature manipulations have no significant effect on standard length, tail length, head length and body area of this species.

5.1 Effects on survival and growth

In the present study, only one fish in high and another one in ambient temperature treatment died during the experimental period. Since the experimental temperatures were not severely stressful to the reared fish (information of through trials), they managed to cope with the manipulated environment and therefore, I found no significant mortality in this study. Similar findings were also found by Ogunji *et al.*, (2017); Hesni *et al.*, (2009) and Selong *et al.*, (2001). where they demonstrated that these rearing temperatures were not too harmful to kill their experimental fish. Ogunji *et al.* (2017) reported that 25 - 28°C was a suitable temperature range that was appropriate for growth of *Clarias gariepinus* (African catfish). In the study they found that fingerlings were not able to survive when water temperature reached to 40°C. During this extreme temperature, *C. gariepinus* failed to adapt and were unable to respond physiologically. They concluded that this results in mortality was caused by changes in the metabolic pathways and collapse in osmoregulatory functions. Another experiment was carried out by Hesni (2009) for the effects of temperatures (15, 20, 25 and 30°C) on survival and moulting of the narrow-clawed crayfish (*Astacus leptodactylus*). They found that at 15°C there was no mortality, while moulting occurred at a lower rate. Selong *et al.* (2001) investigated the effect of temperature on survival and growth of bull trout. They revealed that survival of age-0 bull trout was at least 98% at 8, 10, 12, 14, 16 and 18°C, but 0% at 22, 24, 26 and 28°C after 60d. All fish died prior to reaching 28°C and the predicted ultimate upper incipient lethal temperature for those trout was 20.9°C.

However, some findings of previous works are not consistent with the results of present study (Marcela *et al.*, 2011; Rajinder *et al.*, 2017; Zhijing *et al.*, 2015). An experiment was conducted by Marcela (2011) to study the effect of four water temperatures (15, 20, 25 and 30°C) on survival and growth rate of juvenile *Macrobrachium borellii*. Author mentioned that the highest survival rate was found at 20°C (88%) and 25°C (96%) and the lowest survival at 15°C. Zhijing (2015) revealed that temperature could affect the survival, growth and body composition of *Plectropomus leopardus* where different water temperatures (e.g. 15, 20, 25, 30 and 35°C) were maintained to rear the experimental fish. They found that *P. leopardus* had greater survival rate at 35°C than at 15°C. The findings of these previous experiments are not consistent with the results of present study because of species differentiation and variation of their thermal tolerance.

The present study revealed significant impacts of experimentally manipulated temperatures on body weight, whereas no significant effects were found on other growth parameters (e.g. standard length, tail length, head length and body area). The ultimate result showed that fish kept in low temperature had significantly higher weight than the high and ambient treatment group (figure 7). This finding is consistent with some previous works (Pandit *et al.*, 2010; Stewart *et al.*, 2014). Pandit and Nakamura (2010) reared two age groups of female Nile tilapia (9 and 50 days after hatching) at 27, 32, 35 and 37°C water temperatures for 45-60 days. At the end of study, total weight, total length and FCR of both age groups of fishes were significantly reduced at 35 and 37°C compared to 27 and 32°C ($p < 0.05$). They noticed that higher temperature ($> 32^\circ\text{C}$) resulted in slow growth and reduced feeding efficiency. In another study, Stewart *et al.* (2014) revealed that temperature could affect the growth of channel catfish (*Ictalurus punctatus*) where different water temperatures (27–31°C or 32–36°C) were maintained to rear the experimental fish. They found that channel catfish had greater growth in weight or length at 27–31°C than at 32–36°C. Thus, all these previous experiments have revealed that growth of different fish species was temperature dependent especially growth was better in low temperature because of less stress, physiologically suitable through modulation of metabolism, increasing feeding efficiency, regulating hormones etc. which are consistent with the results of present study. On the other hand, some findings are not consistent with the results of present study (Kausar *et al.*, 2006; Hundt *et al.*, 2015; Nguyen *et al.*, 2015). Kausar *et al.* (2006) reported that the Indian major carp (*Labeo rohita*) has an optimum growing temperature in the range of 25-30°C at which they grow quickly.

Growth rate of this fish decreased during the low water temperature period. Hundt *et al.* (2015) conducted a study with allis shad (*Alosa alosa*) rearing at 16, 20, 24 and 28°C for ten days and revealed that the highest growth (in length) was at 28°C, while it was minimal at 16°C. Another research was done by Nguyen (2015) to determine the effect of temperature [24, current ambient (27-28°C), 30, 32, 34, and 36°C] on the growth performance in cultured Tra catfish (*Pangasianodon hypophthalmus*). Nguyen (2015) mentioned that low temperature (24°C) significantly affected fish growth rate and FCR value, while high sublethal temperature led to enhanced growth performance until 34°C where it peaked. The findings of these previous experiments are not consistent with the results of present study because of experimental design, different species, various temperature ranges, etc.

5.2 Effect on colour pattern

As with the growth performance, I have found that temperature manipulations had significant effects on overall colour of stripped dwarf catfish. The result has revealed that high treatment group had significantly more bright/dark coloured fish compared to the ambient and low treatment groups (see figure 8). On the other hand, ambient treatment group had significantly higher number of normal/natural coloured fish than their opponent group. Although no study was conducted to explore the effect of temperature on colour patterns of *M. vittatus*. Only, a few studies were carried out to unveil how temperature variation can influence colour patterns in other species (e.g. Fanta *et al.*, 1997; Keller *et al.*, 1995; Biedermann *et al.*, 1992; Hoffmann *et al.*, 1990; Parker *et al.*, 1904). Author reported that the colour of frillfin goby (*Bathygobius soporator*) fish was mainly light colour with spots in different levels of temperatures (18°C and 28°C). But there was a tendency of becoming dark fish at low temperature (Fanta, 1997). Keller (1995) observed that when a chameleon (African) was subjected to heat at 30° to 38° it took on a lighter tint than usual and retained this even under strong illumination, where it would be expected to become dark. Another research was done by Biedermann (1992), who experimented on the chromatophores of frogs and tree-toads, fully confirmed the earlier observations of Hering (1969) to the effect that in cold water the pigment of the melanophores passed out into the cell processes, producing a dark skin, and in warm water it passed back into the body of the cell, leaving the skin light. Hoffmann (1990) revealed that the dark skin of *Phrynosoma herandesi* on cool, cloudy days, and its pale skin

on sunny days might be due to temperature differences. Parker *et al.* (1904) showed that the rate of the colour changes in *Anolis carolinensis* was always more or less influenced by temperature. They mentioned that at 10°C *A. carolinensis* became brown and remained irrespective of illumination. At 40–45°C, they became green or greenish-gray, and remained so irrespective of illumination. So, temperature has a great influence on the expression of colour patterns in many organisms that the present study has also revealed.

5.3 Effects on egg and sperm number

The study has revealed that temperature manipulation had significant effect on egg number where both the low and ambient treatment group fish possessed significantly higher number of eggs than the high treatment group (see figure 9). This present work is consistent with a previous work in which Faruk *et al.* (2012) reported that egg production and growth of monosex Nile tilapia (*Oreochromis niloticus*) were maximum at 25°C, while they were minimum at observed at 33°C. Another research was done by Arantes *et al.* (2011) to observe the influence of water temperature on induced reproduction by hypophysation and final oocyte maturation of the *Prochilodus argenteus* which is not consistent with the results of present study. They revealed that by manipulating water temperature alone, spawning performance of *P. argenteus* was significantly different for the two temperature regimes, such that spawning of the fish kept at 23°C (group A) was delayed by 2 h in relation to those kept at 26°C (group B). Besides the delay in spawning, it was also noted that 33% of fish in A group did not ovulate and consequently did not spawn, whereas in group B, spawning occurred for all females.

On the other hand, the study has showed a significant variation in sperm number among different treatment groups where low treatment group fish had significantly higher number of sperm than the high and ambient treatment group (see figure 10). The result is consistent with some previous works (Fenkes *et al.*, 2017; K. Dumorne *et al.*, 2018; Breckels *et al.*, 2013). Fenkes *et al.* (2017) investigated the thermal impacts on gamete quality in brown trout (*Salmo trutta*) and mentioned that early in the season, warm acclimation of males reduced their fertilization probability (lower sperm velocity) when compared with cold- acclimated males. Another research was done by Breckels *et al.* (2013) on the effects of elevated temperature on the sexual traits where guppies

were exposed from birth to one of four temperature treatments: 23, 25 (control), 28 or 30°C. Breckels *et al.* (2013) reported that increased in temperature resulted in lower quantity sperm.

Dumorne *et al.* (2018) reported the effects of pH (6, 7, 8 and 9) and temperature (4, 8 and 16°C) on sperm motility of pink cusk-eel (*Genypterus blacodes*) and revealed that the longest motility duration was recorded at 4°C and the maximum percentage of motile cells was recorded at 8°C. All these previous experiments revealed that the sperm traits are condition dependent which is consistent with the present study.

Chapter-6

6.0 Conclusion:

Environmental and ecological factors can directly influence phenotypes and capable to produce more durable phenotypic variation. Among such factors, temperature has a considerable importance as ecological factors that directly affect the phenotypic traits of aquatic organisms. Overall, the present study revealed that temperature can influence some phenotypic traits in striped dwarf catfish (*Mystus vittatus*). The findings emerged from my work concluded that temperature had a significant effect on most of the selected traits, such as fishes that assigned to the low temperature group exhibited increase in body weight compared to those assigned to the ambient and high treatment groups. Besides, high treatment group has significantly more bright/dark coloured fish than ambient and low treatment. Ambient treatment group has significantly higher number of normal/natural coloured fish than high and low treatment group. I also found that the low treatment group fish possessed significantly higher number of eggs than the high treatment group. The low treatment group fish had significantly higher number of sperm than the high and ambient treatment. However, the study also showed that temperature manipulations had no significant effect on standard length, tail length, head length and body area. The findings from this study suggest that striped dwarf catfishes are likely to provide an ideal and perfect experimental species for testing these possibilities. Moreover further experiments are strongly recommend to expose the effects of environmental manipulations on some important postcopulatory traits (e.g. egg and sperm quality, fertilization, reproductive success and offspring fitness) and transgenerational epigenetic effects using some model species.

Chapter-7

7.0 References:

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