Periodic Effects of Salinity on Compensatory Expression of Phenotypic Traits in Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Salinity fluctuation is a great threat for fish as most species either do not grow well or eventually die in coastal regions where salinity changes happen regularly because of different natural calamities. This study was conducted to explore the effects of periodic changes of salinity on different phenotypic traits in Nile tilapia (*Oreochromis niloticus*) in order to find out any traits that can be expressed through compensatory growth. Equal sized juvenile Nile tilapias were randomly assigned into two treatments including control treatment (CT: constantly 0 ppt) and salinity treatment (ST: 25 ppt in 1st and 3rd month and 0 ppt in 2nd month). Each treatment contained 45 fish having three replications and the entire study was carried out up to three months. After each experimental month, all the selected phenotypic traits (i. e. standard length, tail length, body area and color pattern) were measured. The results revealed that ST fish had significantly smaller standard length and body area compared to CT fish especially after the 1st month of the experiment, while no significant variations were found in other months. The study also showed no significant variations in tail length and color patterns between CT and ST fish throughout the experimental months. The findings revealed that ST fish showed a trend of compensatory growth (i.e. recovery of standard length and body area) after 2nd month of rearing at control condition (0 ppt). The overall results conclude that periodic salinity fluctuation can affect adversely the growth of fish which is an alarming issue for tilapia farmers and other stakeholders. However, the compensatory development of certain traits through phenotypic plasticity can minimize at least these losses.

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INTRODUCTION

Climate change variables including salinity, temperature, pH, dissolved oxygen levels, etc. have significant impacts on fish survival, growth and other physiological activities (Crozier *et al*., 2021; Huang *et al*., 2021; O'Connor and Booth, 2021). Among these factors, salinity is considered very important because of its great influence on several phenotypic traits in many fish (Altinok and Grizzle, 2001; Arjona *et al*., 2008; Overton *et al*., 2008; Küçük, 2013; Rahman *et al*., 2022). For

examples, growth rate of Eurasian perch (*Percafluviatilis*) decreases with increasing salinity (Overton *et al.*, 2008). Fluctuation of periodic salinity affects the expression of growth and color pattern in stripped dwarf catfish (*Mystus vittatus*) (Rahman *et al.*, 2022). Goldfish (*Carassius auratus*) shows better growth performance at 0 to 2 ppt salinity (Luz *et al.*, 2008). Food consumption rate of common carp (*Cyprinus carpio*) decreases by increasing salinities (Wang *et al.*, 1997). Lowering salinity from 39 to 15 ppt in marine water causes stress to marine fishes and decreases feed intake and growth rate (Arjona *et al.*, 2008).

Salinity tolerance is a physiological mechanisms and morphological adaptations that balance concentrations of salts in the cells and tissue of an organism against the external environment. There are many factors that can affect the ability of fish to survive because of increase or decrease in salinity. It can vary between species, populations and to some extent individuals of the same population (Dunlop *et al.*, 2005). The duration of exposure and rate of increase in salinity concentrations may also affect salinity tolerance. A population that has exposed to elevated salinity for longer periods may have evolved greater tolerance than another population of the same species that has exposed to a lower concentration of salinity (James *et al.*, 2003). The tolerance or adaptation with the changing environment by fish is only possible through phenotypic plasticity that occurs when individuals of a given genotype adjust their phenotype according to the conditions they experience (Schlichting and Pigliucci, 1996; West-Eberhard, 2003).

Phenotypic plasticity or compensatory traits describe the tendency of an organism to express different phenotypes (Agrawal, 2001) such as short-term flexible behavior (Ramachandran *et al.*, 1996; Rodrigues *et al.*, 2009), or long-term developmental changes that affect the adult form (Price, 2006) depending on the environment. A wide range of taxa including insects, fish, frogs, birds and mammals show these compensatory phenotypic traits (Crozier and Hutchings, 2013; Merila and Hendry, 2013; Reusch, 2013; Metcalfe and Monaghan, 2001; Portner and Peck, 2010). It allows an animal to adjust in an immediate environmental change and increase its fitness (Van Buskirk, 2012). A lack of phenotypic plasticity can contribute to the exclusion of species.

Likongwe *et al.* (1996) reported that a temperature of 28-32°C and salinity of 0-12 ppt can increase rapid growth of juvenile Nile tilapia. Kangombe and Brown (2008) observed that Nile tilapia shows better growth when increasing the salinity from 5 to 10 ppt in fresh water. In another study, Imsland *et al.* (2008) documented that lowering salinity from 32 to 15 ppt in marine water enhances growth in turbot (*Scophthalmusmaximus*). Nugon (2003) reported that juveniles of *Oreochromis. aureus* and

O. niloticus exhibit good survival in salinity regimes up to 20 ppt, the Mississippi commercial tilapia survived salinity regimes up to 10 ppt but exhibited poor survival at 20 ppt (5%). Wang *et al.* (2001) reported that complete mortality can occur when directly transfer *O. mossambicus* from 0 to above 30ppt. Chervinski and Hering (1973) found *Tilapia zillii* to survive and grow in salinities of about 40 ppt. Another study reported 100% mortality of *T. zillii* upon transfer to 30 ppt salinity (Osborne, 1979).

Salinity fluctuations in the environment may occur due to seasonal rainfall, rates of evaporation, tidal oscillations, flood and other reason and this fluctuated salinity can affect survival of fish by interfering with key physiological and metabolic processes such as different developmental stages, osmoregulation and growth (Gomez-Mestre *et al.*, 2004). The Nile tilapia (*O. niloticus*) is one of the most important vertebrate model species for studies of fish physiology, developmental biology and genetics particularly because of its broad tolerance to an array of environments (Schofield *et al.*, 2011; Grammer *et al.*, 2012; Mathew *et al.*, 2020; Rahman *et al.*, 2022). This species have been observed to exhibit tropic plasticity according to the environment and the other species they coexist with (Bwanika *et al.*, 2007). Therefore, it is very important to know the effect of periodic salinity change on growth and survival of this fish. Several studies on salinity tolerance of tilapias have been conducted (Jennings and Williams, 1992; Suresh and Lin, 1992; Likongwe *et al.*, 1996; Wang *et al.*, 2001; Nugon, 2003; Kangombe and Brown, 2008. But it is not well known whether this species has the capacity to recover from the extreme salinity stress. To best our knowledge, no specific study was conducted to explore whether Nile tilapia exhibits pronounced compensatory growth after periodic salinity stress. Therefore, the present study was carried out to expose the compensatory development of different phenotypic traits due to the periodic salinity stress on Nile tilapia in a controlled laboratory condition.

MATERIALS AND METHODS

Ethical approval

The experimental procedures were approved by the Animal Ethics Committee at Khulna University (Research ref. no.: KUAEC-2017/05/12).

Sample collection and acclimatization

Approximately 200 juveniles of Nile tilapia were collected from a local fish hatchery at Khulna, Bangladesh. The juveniles were then transported in oxygenated poly bag to the Wet Fish Laboratory of Fisheries and Marine Resource Technology (FMRT) Discipline where the

experiment was carried out. Then they were transferred in a large aquarium and conditioned for two days before starting the experiment using the glass aquarium (50cm×29cm×30cm). During this time, fish were fed artificial diet (Progoti Feed Ltd. Bangladesh- 31% protein, 5% fat, 12% moisture and 4% fibre) at a rate of 3% of their body weight.

Water quality management

Prior to stocking of fish, each aquarium was cleaned up thoroughly and filled with tap water. Continuous aeration was ensured throughout the experimental periods. About half of the water was removed by siphoning every day to clean the faces and uneaten food. The dead fish (if any) were removed and recorded in the morning and in the evening prior to feeding. Water quality parameters such as temperature, dissolved oxygen (DO), pH, and salinity were recorded daily using the mercury thermometer, DO meter (Lutron DO-5510) and pH meter (Hanna ISO 9001) and a portable refractometer (RHS-10ATC Handheld 0-10% ATC Salinity Refractometer) respectively. About 30-40% water was replaced twice a week.

Experimental design

The overall design of the experiment is shown in Figure 1. Ninety juveniles of Nile tilapia were randomly assigned into two salinity treatments such as control treatment (hereafter known as CT) and salinity treatment (hereafter called as ST). Each treatment had three replications and 15 fish were randomly stocked in each replication up to three months. CT fish were reared constantly at 0 ppt, while ST fish were reared at 25 ppt during 1st and 3rd month and 0 ppt in 2nd month of the experiment. In ST, salinity was increased or decreased gradually (±2 ppt/day) to avoid any salinity stress. The fish were fed the locally available commercial supplementary colorless fish feed (Progoti Feed) every day morning at a rate of 3% of their body weight.

Determination of growth and survival

Before starting the experiment, individual juvenile was anaesthetized using the ice bath and then the standard length (the distance in cm from the snout to the tip of its caudal peduncle) was measured to closest 0.1 cm by using a measuring board. After every month, each fish was anaesthetized using the ice bath. Then a photograph was captured by using a digital camera (Canon DS126621) when a laminated graph paper was placed under each fish. The camera was set with a strand to take the photograph of the fish individually. From the raw images, standard length, tail length (the distance in cm from the caudal peduncle to the end of its caudal fin) and body area (the

area (cm^2) of the body excluding all fins), were measured by using *ImageJ* software (version 1.50i). Fish mortality was also monitored and recorded for each tank regularly.
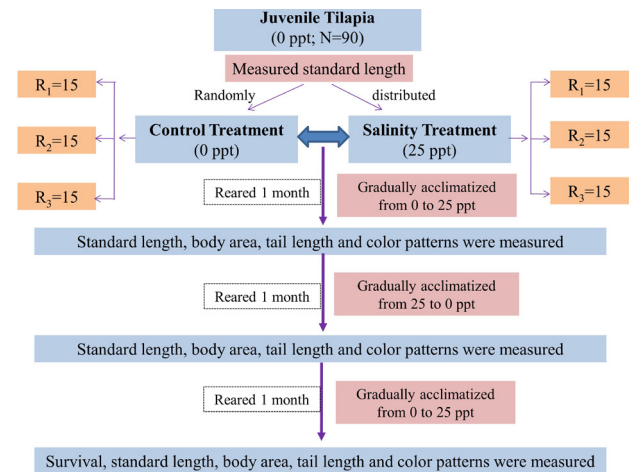

Fig. 1. The overall design of the experiment.

Observation of color pattern

Color pattern of individual fish at different experimental periods was visually observed and recorded from the captured raw images. There were three different colors of tilapia observed during the experimental periods such as dark/black color, normal/natural color and fade/pale color (Fig. 2).
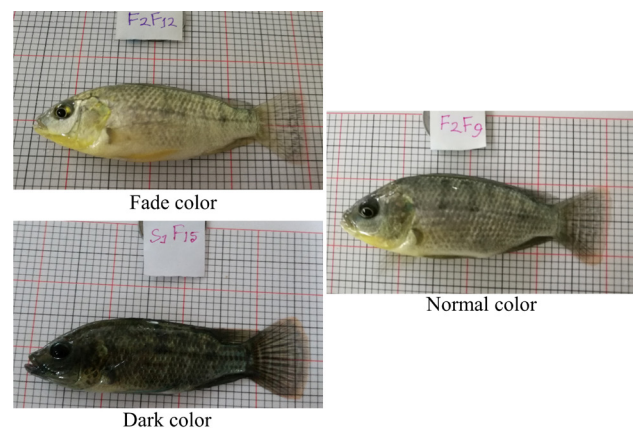
Fig. 2. Types of observed body color in experimental *Oreochromis niloticus.*

Statistical analyses

All analyses were performed using 'RStudio' version 4.0.2 (R Development Core Team, 2020). 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). First, all traits were tested to check their

normal distribution and then appropriate transformations were applied to yield normal distributions for non-normal distributed traits.

The one-way analyses of variance (ANOVA) models using the 'car' package was performed to explore the effect of salinity treatments on the expression of different phenotypic traits (e.g. standard length, tail length, body area, color patterns, etc.) throughout the experimental period. The subsequent post-hoc tests were also conducted to do multiple comparisons through tukey contrasts using the multcomp package (Hothorn *et al.*, 2008). To explore the color patterns (i.e. categorical data), the Pearson's chi-squared test was performed using gmodels packages (Warnes *et al.*, 2015). Cramer's V based on adjusting chi-square significance was also calculated using 'vcd' package (Meyer *et al.*, 2016) to find out the association between color patterns as a percentage of their maximum possible variation. All graphs were made using ggplot2 package (Wickham, 2009).

RESULTS

Effects on growth and survival

Standard length (SL)

The standard length of fish reared in both CT and ST) for three successive months are presented in Figure 3. At the beginning of the experiment (0-month at 0 ppt), there was no difference in SL ($F_{1,88} = 0.22$, p=0.64) between two treatments. After one month of rearing ST fish at 25 ppt and CT fish at 0 ppt, both CT ($F_{1,88} = 43.19$, p<0.001) and ST ($F_{1,88} = 43.63$, p<0.001) fish obtained significant SL than their initial SL. The comparison of SL between CT and ST after one month showed that CT fish were significantly larger than that of ST ($F_{1,88} = 3.79$, p<0.05). In 2nd experimental month, both CT and ST fish were kept in control conditions (0 ppt) up to one month. No significant variation was found in SL between 1st and 2nd month of CT fish ($F_{1,88} = 3.05$, p=0.08), while a significant difference was found between ST fish ($F_{1,88} = 8.79$, p<0.01). The comparison between CT and ST fish revealed no significant variation in SL during 2nd month ($F_{1,88} = 0.33$, p=0.57). In the last month of this experiment, ST fish was again reared at 25 ppt for one month, while CT group was constantly kept at 0 ppt. After one month, the results revealed a significant variation in SL between 2nd and 3rd month of CT fish ($F_{1,88} = 7.56$, p<0.01), no significant difference was found between ST fish ($F_{1,88} = 2.89$, p=0.09). The comparison between CT and ST fish after 3rd experimental month revealed no significant variation in SL ($F_{1,88} = 3.14$, p=0.08).

Tail length (TL)

After 1st month of the experiment, the comparison of TL between CT (reared in 0ppt) and ST (reared in 25ppt) showed no significant variation ($F_{1,88} = 1.30$ and p=0.26). Similarly, no significant variations in TL were detected between CT and ST fish during 2nd month ($F_{1,88} = 0.31$ and $P=0.58$) and 3rd month ($F_{1,88} = 2.07$ and $P=0.15$).

Total body area (TBA)

The comparison of TBA between CT (reared in 0ppt) and ST (reared in 25ppt) fish reared up to one month revealed that CT fish had significantly larger TBA than their counter ST fish ($F_{1,88} = 5.56$, p<0.05 and Fig. 4).Unlikely the 1st month of experiment, the comparison between CT and ST fish revealed no significant variation in TBA when both groups were reared in controlled conditions ($F_{1,88} = 0.19$, p=0.66 and Fig. 4).After the 3rd month of experiment, the comparison of TBA between CT and ST groups showed again no significant variation ($F_{1,88} = 2.03$, p=0.16 and Fig. 4). Survival: Throughout the experiment period, this study found 100% fish survival in both CT and ST.
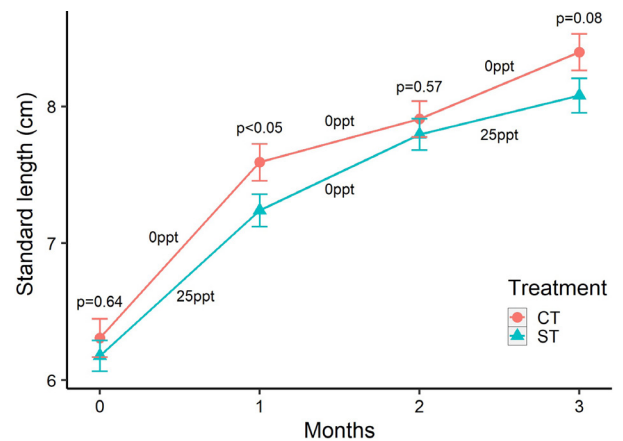

Fig. 3. Variation in standard length (cm) of experimental *Oreochromis niloticus* reared in control treatment (CT) and salinity treatments (ST) up to three months. Values are given as mean ± standard error (SE). The analysis was done using 95% confidence level.

Effects on color patterns

The findings of color variation between CT and ST in different months are presented in Table I.

Dark color

The Pearson's chi-squared test revealed no significant variation in dark coloration between CT and ST fish after one ($\chi^2 = 0.10$ and p=0.75), two ($\chi^2 = 0.96$ and p=0.33) and three ($\chi^2 = 0.0$ and p=1.0) months of rearing.

Normal color

Like dark coloration, no significant variation was

observed in normal coloration between CT and ST fish after one (χ^2=1.12 and p=0.29), two (χ^2=0.46 and p=0.49) and three (χ^2=0.22 and p=0.64) months of rearing.
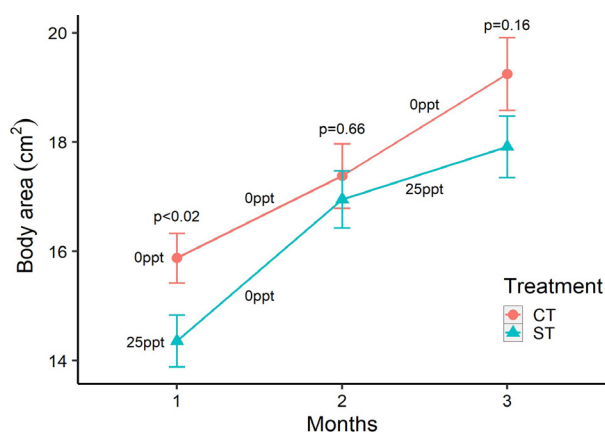

Fig. 4. Variation in total body area (cm^2) of experimental *Oreochromis niloticus* reared in control treatment (CT) and salinity treatments (ST) up to three months. Values are given as mean ± standard error (SE). The analysis was done using 95% confidence level.

Table I. Outcomes of Pearson's chi-squared test to explore the color patterns at different experimental periods between control treatment (CT) and salinity treatment (ST).

Month	Treatment	Color patterns with number of fish			χ^2	p
		Normal/ natural	Dark/ black	Fade/ pale		
1st	CT (0 ppt)	18	5	22	0.10	0.75
	ST (25 ppt)	23	6	16		
2nd	CT (0 ppt)	18	9	18	0.96	0.33
	ST (0 ppt)	16	13	16		
3rd	CT (0 ppt)	12	14	19	0.0	1
	ST (25 ppt)	14	15	16		

Fade colour

The Pearson's chi-squared test revealed no significant variation in fade coloration between CT and ST fish after one (χ^2=1.64 and p=0.20), two (χ^2=0.46 and p=0.49) and three (^{2}=0.42 and p=0.52) months of rearing.

DISCUSSION

Species specific salinity tolerance is vital for commercial culture of fish in areas where salinity fluctuate abruptly and/or periodically. In coastal regions of tropical areas salinity changes happen regularly and therefore, it is important to explore suitable fish species that can tolerate the any changes of salinity without compromising the growth and survival. In the present study, standard length and total body area of experimental Nile tilapia juveniles were significantly decreased in 1st month when ST fish were reared at 25 ppt. These findings corroborate the results documented by Luz *et al.* (2008), Lawson and Anetekhai (2011), Kalio (1988), etc. Lawson and Anetekhai (2011) found that total length of Nile tilapia decreased as salinity increased. Similar to the present study, McGeachin *et al.* (1987) found that the growth rate of *O. aureus* sharply decreased at 36 ppt. They showed that total length of Nile tilapia increased about 30% and 10% at 0 and 7 ppt, respectively. In another study conducted by Kalio (1988) found increasing the salinity from 25 ppt to 50 ppt that growth of Nile tilapia at high salinity was significantly lower than that in freshwater, whereas survival was not affected by salinity. This study also found that changing salinity has no significant adverse effect on fish survival throughout the experimental periods.

Fishes are living in water need exact concentration of certain substances in water (Moyle and Cech, 1982). Fresh water fishes have high concentration of salt in their body compared to the surrounding water. But when they suddenly exposed to high salinity, water comes out of the body by osmosis and fish can suffer from water deficiency (Wilson, 2011). The rate of osmosis depends on body surface area and concentration gradient. In the present study, ST fish showed a trend of salinity tolerance or adaptation through osmoregulation. When ST fish were gradually exposed to the higher salinity (25 ppt), the growth (SL and body area) significantly decreased compared to CT fish and control condition (0 ppt). But when the ST fish were reared gradually to 0 ppt, their osmoregulatory process switched, i.e. they required less energy to adapt with the freshwater (Kombat *et al.*, 2021; Rahmah *et al.*, 2020) and thereby, they saved the energy that was used for osmosis. Thus, ST fish reared at 0 ppt showed significant increase of SL and body area as a compensatory growth and after one month, they attained almost the same growth as CT fish.

Fish tail (caudal fin) is an important organ to propel its body through water and to move swiftly. Spurný (1998) observed that fish without caudal fin required 40% more energy for movement and its movement was not fluent. It is also the indication of fish health. In the present study, no significant variation of tail length was observed between ST and CT fish throughout the experimental months. This finding together with survival results indicate that the experimental fish were not stressed too much under salinity treatment to compromise with these traits.

Fish body color is an imperative phenotypic trait and

considered as a tool for predator escaping (camouflage, bartesian mimicry and palatability signal), prey capture (camouflage, aggressive mimicry) and conspecific communication (mating and agonistic signaling, shoaling preferences) (Cheney *et al*., 2008; Mills and Patterson, 2009). Body coloration is also influential in contribution to market price and consumer choice (Yilmaz and Ergün, 2011; Mustapha *et al*., 2012). Although color variations between the treatments in the present study were insignificant, three different color of experimental Nile tilapias were observed. Some studies showed that fishes could change their body color for environmental (e.g. salinity, temperature, habitat, etc.) changes (Fanta, 1997; Fanta *et al*., 1995; Briand *et al*., 2005; Rahman *et al*., 2019, 2020, 2021). In a study, Fanta (1997) reported that body color of *Bathygobius soporator valenciennes* was more variable when they were exposed to extreme salinity Another study by Fanta *et al*. (1995) found that *Gobionotothen gibberifrons* became darker at high salinity and lighter at low salinity. Rahman *et al*. (2021) found significant effects of periodic salinity variation on color pattern in stripped dwarf catfish. In another study, Rahman *et al*. (2020) showed that male guppy (*Poecilia reticulata*) can modulate their color patterns under different rearing temperatures. Rahman *et al*. (2021) also found an experimental evidence that different light and shelters (PVC pipe) can influence the body color expression in stinging catfish (*Heteropneustes fossilis*). Thus, studied suggest that at stressed condition, generally light color fish becomes darker and dark color fish becomes lighter (Fanta-Feofiloff *et al*., 1983; Fanta *et al*., 1989a, b; Pickering, 1981). Briand *et al*. (2005) studied on glass eel (*Anguilla anguilla*) and observed less advanced pigment at higher salinity (0 ppt to 29.2 ppt). Similar finding was also obtained by Briand *et al*. (2005). As mentioned above, perhaps experimental Nile tilapia reared in different salinities were not stressed too much in this experiment which could have such type of effect on their color patterns.

CONCLUSION

Salinity is one of the most influential aquatic factors that can affect directly or indirectly some phenotypic traits and physiological activities of different fish species. The findings of the present study showed that periodic changes of salinity significantly affect some growth parameters (e.g. SL and body area) of the commercially important Nile tilapia. However, they could compensate this growth when they reared again to the control condition. This is an important information to the farmers and other stakeholders who can reduce their severe losses when they face any salinity change issues in their tilapia farms.

Although periodic salinity changes did not show any significant effects on survival and color patterns, some other important traits related to reproduction and offspring fitness should be studied in the future.

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