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Full Length Article

Effects of Different Rearing Temperatures on the Expression of Some Phenotypic Traits in a Freshwater Catfish (*Mystus vittatus*)

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Abstract

Global warming is the most concerning issue in the recent years which directly and indirectly influences many fish species enforcing to modulate their different phenotypic traits to cope with the unprecedented conditions. This study was conducted to explore the expression of some phenotypic traits of a popular small freshwater fish, the stripped dwarf catfish (*Mystus vittatus*), reared for 30 days under different water temperatures such as 1) control (26–28ºC), 2) high (30–32ºC) and 3) low (20–22ºC). The principal component analysis for six growth parameters revealed the formation of different clusters because of temperature effects. The respective individual analysis showed that high temperature reared fish had significantly lesser body weight than the low treatment, and marginally lower than the control treatment. The study revealed that significantly higher number of fish reared in control treatment possessed natural body colour than high treatment, while high treatment had significantly larger number of dark coloured fish than the control one. Taken all together, the research outcomes of this work show the sensitivity of some phenotypic traits of *M. vittatus* at different temperatures and the information should be taken into account for their potential rearing, future sustainable aquaculture and conservation. © 2022 Friends Science Publishers

Keywords: Global warming; Catfish; Fish morphology; Fish colour

Introduction

Recently a meta-analysis reported that extinctions of 47% of the 976 species surveyed locally could be happened across the globe during the 20th century because of the impacts of climate change (Wiens 2016). Although all ecosystems were affected by these climate changes, the frequency of local extinctions in freshwater habitats was significantly higher (75%) than the marine (51%) and terrestrial (46%) habitats. Evidence from different studies also confirmed that freshwater ecosystems are the most threatened inhabitants in the planet where many important species live (Döll and Zhang 2010).

Fish are the most vulnerable species amongst all the living organisms in the freshwater ecosystems (Nyboer *et al*. 2019). Because of their ectothermic conditions, fish are very sensitive to different stresses caused by temperature and other water parameters related changes which ultimately induce their physiological activities (Lapointe *et al*. 2018) and thereby, affect their survival, behaviour, growth, reproduction and production (Pankhurst and Munday 2011; Mazumder *et al*. 2015; Comte and Olden 2017; Funge-Smith 2018). Studies showed that temperature rising due to the global climate change is the main concerning issue which can directly or indirectly alter freshwater fish habitats (Mulholland *et al*. 1997), influence their food webs (Gibert 2019), increase mortality (Till *et al*. 2019), modulate behavior (O'Gorman *et al*. 2016) and affect growth and reproduction (Pankhurst and Munday 2011; Eldridge *et al*. 2015).

Inland freshwater fish are the major fisheries commodities in the South Asian countries and this region alone produced 22.6% of the total global inland fisheries production during 2015 (Funge-Smith 2018). These freshwater fisheries resources are considered as the most important sources of food security and protein supply for many of the developing countries in tropical regions (Béné *et al*. 2015). People living in these tropical countries obtain major amounts of their total dietary protein and nutrients mainly by the intake of freshwater fish (Funge-Smith and Bennett 2019). Moreover, their livelihoods as well as the economy of their areas broadly depend on these valuable fisheries resources (Welcomme *et al*. 2010; Funge-Smith and Bennett 2019). Unfortunately, these resources are at

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high risk because of the detrimental effect of unprecedented climatic variations, especially global temperature rising (Welcomme *et al*. 2010; Das *et al*. 2013; Barange *et al*. 2018). It has been unveiled that the temperature trend in the South Asian region has been consistently rising in the last few decades (Kumar *et al*. 2011). The projected summer temperature in the South Asian region could be increased by 3°C to nearly 6°C above the baseline by 2100 (Vinke *et al*. 2017). Thus, this rising temperature may have significant effects on the existing fisheries resources in this region. Considering this concerning issue, the present study was carried out to investigate whether different rearing temperatures could affect some important phenotypic traits (*e.g*., growth parameters, body colour and reproductive status) of a popular small freshwater fish, the stripped dwarf catfish (*Mystus vittatus*), under controlled laboratory conditions.

M. vittatus are native to freshwaters and some brackish waters of India, Bangladesh, Myanmar, Nepal, Sri Lanka and Thailand (Hossain 2014; Froese and Pauly 2017). They are mostly found in freshwater bodies especially rivers, canals, lakes, ponds, inundated paddy and jute fields and floodplain areas during rainy season (Froese and Pauly 2017). Their size (standard length) can be varied from 4.30–8.70 cm (Hossain *et al*. 2006). Their body colour varies from gray-silvery to shining golden along with pale blue or dark brown to deep black longitudinal strips on both sides of the body (Froese and Pauly 2017). Because of their delicate colour patterns, they are also becoming as a popular ornamental fish species in this region (Biswas *et al*. 2015). People prefer them for their very good taste, high nutrient value, and their availability throughout the year (Hossain *et al*. 2006; Paul *et al*. 2019). They are enriched with different kinds of fatty acids, amino acids, vitamins and minerals (Paul *et al*. 2019). Considering their popularity, market demands and potentiality, several studies were conducted about their induced breeding (Islam *et al*. 2012), food and feeding (Arunachalam and Reddy 1979) and their importance in livelihood security (Hossain *et al*. 2015) which implied the necessity of their commercial culture. Unfortunately, they are enlisted as a vulnerable and threatened fish species which urges their conservation as well (Hossain 2014). Considering the impacts of elevated temperature on fish phenotypic traits, this study was designed to experimentally explore how extreme temperatures could affect some life-history traits of an important freshwater species, *M. vittatus*.

Materials and Methods

Fish husbandry

Two hundred (200) stripped dwarf catfish (*M. vittatus*) were obtained from the local fish farmers which were immediately shifted in oxygenated poly bags to the fish rearing facilities at the Fisheries and Marine Resource Technology Discipline, Khulna University, Bangladesh. In the laboratory, they were stocked in large glass aquariums (80 cm × 40 cm × 30 cm) and allowed them for conditioning up to one week.

Experimental set-up

After one week of acclimatization, almost same sized 90 healthy *M. vittatus* (body weight: 8.79 ± 0.24 g and ANOVA: $F_{2,87} = 2.04$, $P = 0.14$) were finally sorted out for the experiment. The selected fish were randomly assigned into three temperature treatments such as 1) control (26–28ºC), 2) high (30–32ºC) and 3) low (20–22ºC) for 30 days of the experimental period. Each treatment had two replications where each replication contained 15 fish. The high and low temperature was chosen based on the annual mean daily maximum (31.3ºC) and minimum (21.9ºC) temperature in this region during the summer season (Yu *et al*. 2019). The experiment was carried out in a temperature-controlled room where selected temperatures of all treatments were maintained by using thermostats (Shenzhen Yago Technology Co. Ltd., China).

Feeding and water quality management

The fish were fed once daily up to their satiation level providing a mixture of chopped fish larvae and tiny shrimp flesh along with a small proportion of commercial fish flake (the Hartz Mountain Corporation, Secaucus, NJ). Approximately fifty percent of water from each of the aquarium was replaced and uneaten food, faeces and dirt attached inside the aquarium walls were removed by siphoning weekly. Temperature reading was taken from the digital thermostat as well as with a Celsius thermometer (Alcohol Filled Student Thermometer, GSC International Inc., USA) too to compare the accuracy. During the experimental period, dissolved oxygen (DO) was monitored once daily with a digital DO meter (PDO-519, Lutron Electronic Enterprise co. Ltd, Taiwan) which was within the range of 4.8–6.5 mg/L. Water pH of each aquarium was also monitored once daily using a portable pH meter (Atago Digital pH Meter DPH-2, Atago co. Ltd, Japan) which was also in the range of 7.05 – 7.68.

Survival and growth measurements

Fish was monitored every day and thereby, the number of dead fish was recorded for the calculation of survival rate. At the end of rearing period, randomly each fish was anesthetized for a while using MS222 (Tricaine methanesulfonate). The anesthetized fish was then placed on a laminated graph paper and captured a photograph maintaining a fixed distance (30 cm) using a digital camera (Canon DS126621). Each image included a unique code so that the subsequent analyses of male traits were performed blind of treatment. The *ImageJ* software (v. 1.46) was used for the determination of specific phenotypic traits. Phenotypic traits included total length (TL), standard length (SL), head length (HL), tail length (TLL), body area (BA) were measured cautiously to the nearest 0.1 cm. Finally, individual's body weight (BW) was measured using a digital balance to the nearest 0.1 g. (GF-300, A&D company Ltd., Massachusetts, USA).

Body color observation

Coloration pattern of each fish was recorded cautiously prior to photograph (discussed above). Captured photograph of each individual was then cross-checked to confirm its recorded body colour. The fish colour were classified as natural (Fig. 1A), dark (Fig. 1B) and others not distinctly dark or natural colour (Fig. 1C) based on their body colour after exposed to various temperature treatments.

Statistical analyses

Data analyses were accomplished with the 'R' software (v. 3.6.1) developed by R Development Core Team 2019. Descriptive data (mean ± SE) were evaluated using the 'pysch' package. The Shapiro-Wilk test of normality and the Levene's tests for homogeneity of variance were done with the 'one way tests' package. All data (except body weight) were not normally distributed by any transformation methods. The square root was appropriate for normalization of body weight. The categorical data (body colour) were not used for normalization as they were considered for other respective models (see below).

First, the multivariate model of principal component analysis (PCA) was performed using the 'FactoMineR' package to expose the effect of rearing temperature on the growth performances (TL, SL. HL, TLL, BA and BW). The first three principal components (PCs) were retained finally as they collectively explained 85.92% of the overall variance where each PC had eigenvalue of > 1. The remaining PCs were excluded from the final analysis because of their low explanatory potential (< 7%) and lower eigenvalue (< 0.5).

The univariate analysis of variance (ANOVA) model was performed using the 'car' package, the default Kruskal-Wallis (K-W) test was applied when any variable was not normally distributed even after any transformation (but homogeneous), and the one-way ANOVA with Welch's correction or Welch test (W-T) was applied using the 'one way tests' package when a variable was not normally distributed as well as not homogenized. In the model, each measured trait was included as a 'response variable' and temperature treatment was incorporated as an 'independent factor'. When the significant effect of rearing temperature was revealed, the subsequent post-hoc tests were conducted to explore the multi-comparison among tested trials.

Since the data of body colour (categorical data) did not comply with the assumptions of any parametric model, the Pearson's chi-squared test with Yates' continuity correction was applied using 'gmodels' and 'rcompanion' packages in order to find out the variation in these traits between treatments. During the analysis, the categorical data was included separately into their respective model as a 'response variable', while temperature treatment was fixed as an 'explanatory variable'.

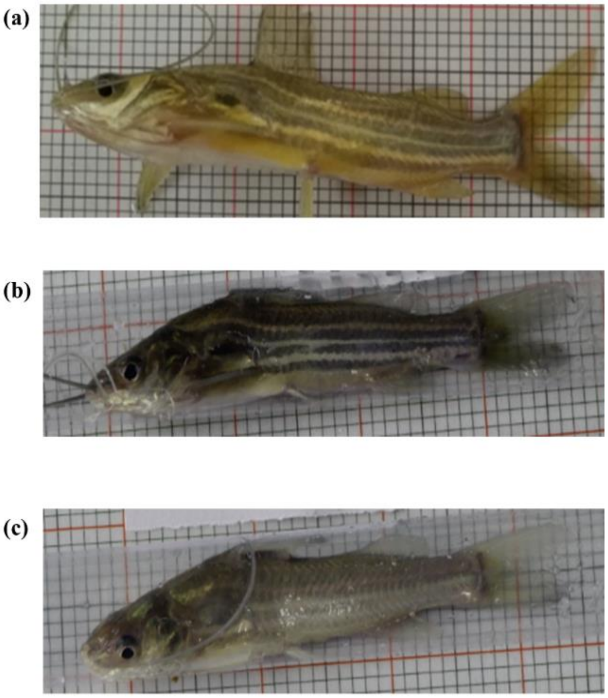

Fig. 1: Types of observed body colour of experimental *Mystus vittatus* reared under different temperatures up to one month. Here, (**a**) natural body colour, (**b**) dark body colour and (**c**) other body colour- not distinctly dark or natural colour (intermediate or confused colour)

To evaluate the magnitude of impacts of rearing temperature, the effect size was determined according to the statistical significance tests developed by Cohen (1988). Finally, all plots were arranged by using the 'ggplot2' package to show the significant variations among treatments graphically.

Results

Survival and growth measurements

During the entire experimental period, only one fish died in control and low temperature treatment groups, while none was found dead in high temperature treatment.

The PCA analysis showed different cluster formations of the measured six growth parameters because of the variation in rearing temperature. The analysis revealed that high temperature fish were characterized by mainly length parameters (*e.g.*, TL, SL, HL and TLL in PC_1), while control and low treatments fish were attributed by BA (PC_2)

Table 1: The effects of rearing temperature on measured growth parameters of the experimental *Mystus vittatus*. Different superscripts of letter indicate statistically significant variations in the respected trait of fish among the treatments ($P < 0.05$)

Response trait	Temperature treatment (mean ± SE)			test-stat	*P*	Effect size	Model
	Control (26-28°C)	High (30-32°C)	Low (20-22°C)				
Total length (cm)	9.51 ± 0.14^a	10.06 ± 0.29^a	9.65 ± 0.17^a	1.46	0.24	f = 0.21	W-T
Standard length (cm)	7.65 ± 0.12^a	8.19 ± 0.25^a	7.84 ± 0.14^a	1.99	0.15	f = 0.23	W-T
Head length (cm)	2.22 ± 0.20^a	2.14 ± 0.07^a	2.17 ± 0.05^a	1.21	0.31	f = 0.14	W-T
Tail length (cm)	1.82 ± 0.03^a	1.91 ± 0.07^a	1.88 ± 0.04^a	3.84	0.15	$\varepsilon^2 = 0.04$	K-W
Body area (cm^2)	14.00 ± 0.42^a	14.18 ± 0.38^a	13.12 ± 0.42^a	1.95	0.15	f = 0.21	ANOVA
Body weight (g)	8.55 ± 0.39^{ab}	7.42 ± 0.30^a	9.14 ± 0.36^b	6.10	< 0.01	f = 0.38	ANOVA

Different superscripts of letter indicate statistically significant variations in the respected trait of fish among the treatments ($P < 0.05$)

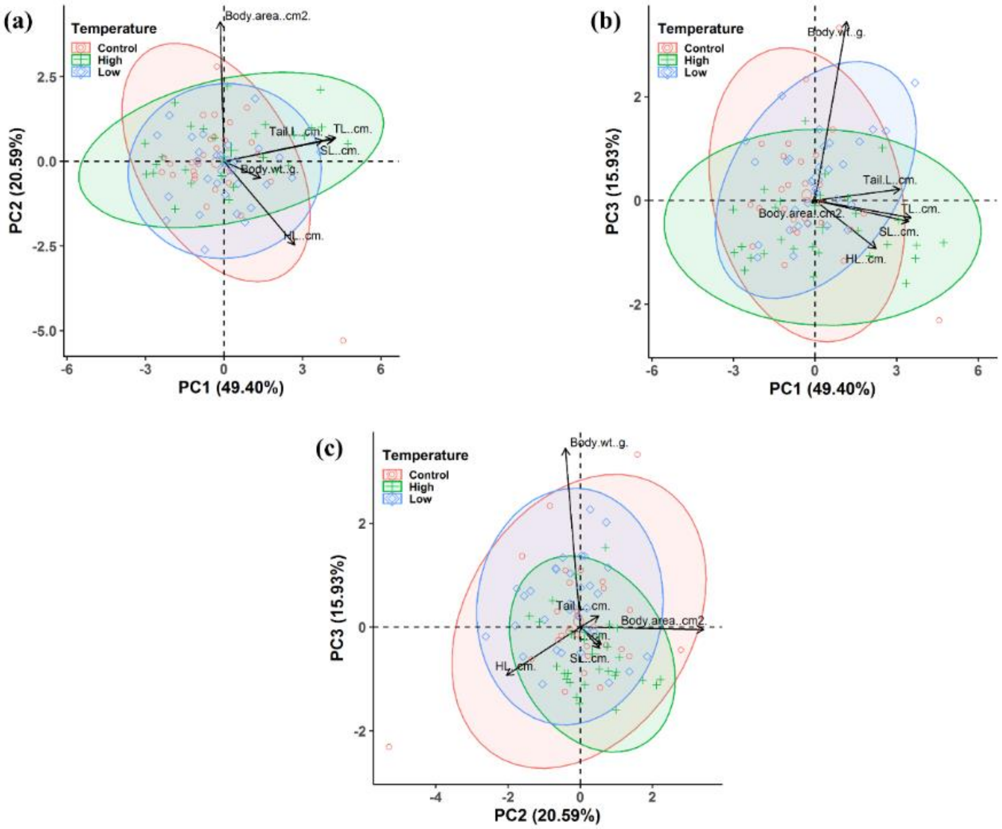

Fig. 2: Biplots of principal component analysis (PCA) for the measured six growth parameters. The plots show how six growth parameters of the experimental *Mystus vittatus* formed clusters because of the effects of rearing temperature. (**a**) PC$_1$ and PC$_2$ explained collectively explained 69.99%, (**b**) PC$_1$ and PC$_3$ explained collectively 65.33% and (**c**) PC$_2$ and PC$_3$ explained collectively 36.53% of the total variance

and BW (PC$_3$) (Fig. 2A–C).

The individual statistical results noticed a considerable influence of rearing temperatures only on the final body weight (Table 1). The subsequent post-hoc tests followed by Tukey HSD revealed that the high treatment reared fish had significantly reduced body weight than the low treatment ($P < 0.01$), while a marginally significant lower body weight was found than the control treatment (P = 0.06). The analysis also revealed no significant variation in body weight between low and control treatment (P = 0.46). Except body weight, other phenotypic traits did not show any significant variation among the treatments (Table 1).

Body color observation

The Pearson's Chi-squared test with Yates' continuity correction discerned that the rearing temperature could influence significantly the fish natural body colour (χ^2 = 9.49, $P < 0.01$, Fig. 3 and Cramer's V = 0.27). The subsequent analysis revealed that control treatment had significantly higher number of natural coloured fish (n =22) than high treatment (n = 11 and $P < 0.01$), while no significant variation was found with low treatment (n=20, P = 0.62 and Fig. 3). There was a marginally significant variation in the availability of natural coloured fish found

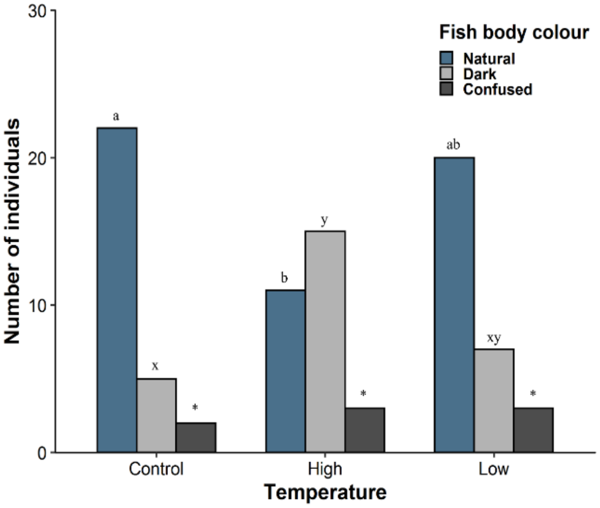

Fig. 3: Number of experimental *Mystus vittatus* possessed different body colours after rearing in different temperatures. Different capital letters indicate statistically significant variations in natural body colour among the treatments ($P < 0.05$), different small letters denote statistically significant variations in dark colour of fish among the treatments ($P < 0.05$) and an asterisk symbol indicates statistically non-significant variation in other colour of fish among the treatments ($P < 0.05$)

between high and low treatment groups ($P = 0.05$).

The findings revealed that dark body coloration was modulated because of the effect of rearing temperature ($\chi^2 = 9.26$, $P < 0.01$, Fig. 3 and Cramer's V = 0.25). Further analysis showed that high treatment had significantly higher number of dark coloured fish (n = 15) than control treatment (n = 5 and $P < 0.05$), while a marginally significant variation was found with low treatment (n = 7, $P = 0.05$ and Fig. 3). However, no significant variation in dark body colour was found between control and low treatments ($P = 0.79$).

Only a very few and insignificant number of other coloured fish (*i.e.*, not distinctly dark or natural colour) was observed in the experimental groups such as two in control treatment, while three in each of low and high treatments (Fig. 3).

Discussion

Global water temperature is undoubtedly rising which ultimately may affect the living aquatic organisms particularly many fish species directly or indirectly in different ways (Crozier and Hutchings 2014; Merilä and Hendry 2014). The elevated water temperature can significantly affect fish behavior (Colchen *et al*. 2016), growth (Islam *et al*. 2019), reproduction (Donelson *et al*. 2014), immunity (Kim *et al.* 2019) and their survival (Crossin *et al*. 2008). Although several studies have been conducted on the effects of elevated temperature on different fish species, unfortunately very few studies have been carried out yet on commercially important freshwater catfish like *M. vittatus* to know their responses to the extreme rearing temperatures (Buentello *et al*. 2000; Tang *et al*. 2000; Arnold *et al*. 2013; Ogunji and Awoke 2017).

The study found no significant effect of rearing temperature variations on survival rate of the experimental *M. vittatus*. Although, no study has been carried out yet with this species on this particular issue, some studies with other tropical freshwater fishes corroborate the findings (Stewart *et al.* 2015; Lapointe *et al.* 2018; Islam *et al.* 2019). The results indicated that *M. vittatus* could survive even under the thermal-induced stress condition. In contrasts, studies with other tropical freshwater species have found significant effects of temperature on their survival rates (Tang *et al.* 2000; Rummer *et al*. 2014; Ogunji and Awoke 2017). These different findings recommend that the effect of temperature on survival rate varies according to species and the geographical locations of their habitats.

In the present study, the high temperature reared fish had significantly lower body weight than their counter groups which is consistent with the previous findings of other catfish species. For instance, Islam *et al.* (2019) have reported that final body weight of *Pangasianodon hypophthalmus* was significantly lower at 24 and 36°C than those of 28 and 32°C. In another study, Arnold *et al.* (2013) have shown that juveniles of *Ictalurus punctatus* had significantly increased body weight at moderate temperature (27–31°C) than those of low (23–27°C) and high (31–35°C) temperature regimes. It was noticed in the present study that fish in high temperature were seemed to be tired, sluggish and reluctant to intake feed immediately. Evidence with elevated temperature has confirmed that fish reduced their swimming and locomotion (Yuan *et al*. 2017), feed consumption and utilization (Buentello *et al*. 2000) which ultimately hamper their metabolic activities (Sandersfeld *et al*. 2015) and energy budget (Anacleto *et al*. 2018) required for proper growth and other physiological purposes. This might be a plausible reason to have lower growth parameters in high temperature reared fish than other treatments in the present study.

Although fish in high temperature showed significantly lower body weight, their other measured growth parameters (*e.g.*, different lengths) were same as those of their counter groups. Thermal-induced stress can force the fish to make a resource allocation trade-off by balancing energy budget between growth traits and maintenance (Angilletta *et al*. 2003; Hemmer-Brepson *et al.* 2014). It has been shown that fish exposed to higher temperature expressed significantly more heat shock proteins in the expense of lot of energy (Viant *et al.* 2003; Werner *et al.* 2006). However, this energy expenditure may not always reduce their growth while allocating it more to coping with the thermal stress (Viant *et al.* 2003; Werner *et al.* 2005) and perhaps the similar mechanism might also work in the growth performance (except body weight) of high temperature reared fish in the present study. However, future study to expose the underlying physiological mechanisms should be carried out to reveal this interesting issue.

Fishes around the world possess different colour patterns for various reasons such as providing signals, bearing identity, acquiring social ranking, getting mating success, confirming reproductive periods, responding to stress, *etc.* (Kodric-Brown 1998; Gamble *et al.* 2003; Burmeister *et al.* 2005; Rahman *et al.* 2013; Moran and Fuller 2018; Hemingson *et al.* 2019). These colour patterns of many animals including fish are condition-dependent, and few studies have already revealed that temperature variation can significantly influence the expression of colour patterns in fish (Breckels and Neff 2013; Rahman *et al.* 2020). However, some studies showed fish colour pattern could be modulated because of various stresses caused by light (Rahman *et al.* 2019), salinity (Rahman *et al.* 2022), diet (Rahman *et al.* 2013) and predation (Rahman *et al.* 2021). In the present study, significantly larger number of high temperature reared fish had dark appearance, while most of the control group possessed natural colour, and moderate number of low treatment fish possessed both appearances. This colour variation may indicate the thermal-induced oxidative stress particularly in high and low treatment groups that has already been revealed in discuss fish (Jin *et al.* 2019), goldfish (Lushchak and Bagnyukova 2006) and guppy (Rahman *et al.* 2020). Studies suggest that these colour changes usually occur because of chromatophores which can be regulated by hormones, neurotransmitters, genetic and environmental factors (Burton 2002; Kelsh 2004). It will be, therefore, interesting to know with further research regarding the underlying mechanisms of colour changes due to thermal stress (Ligon and McCartney 2016).

Conclusion

The overall findings of this present study suggest that, like many other fish species, *M. vittatus* is also at high risk because of the unprecedented global warming. Extreme variations in temperature at their natural habitats may lead to modulate their important phenotypic and reproductive traits to cope with the adverse environmental conditions. Since the findings of this present study warn that extreme rearing temperature (high or low) can affect severely some phenotypic traits of *M. vittatus*, necessary steps and strategies should be taken to save, conserve, breed and culture of this valuable species. Furthermore, studies should be carried out to explore the underlying physiological and genetic mechanisms regarding their coping and modulation strategies under the thermal stressed conditions.

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Author Contributions

Yousof Naser Alrashada Conceptualization, investigation, writing review and editing. Md. Moshiur Rahman Conceptualization, methodology, investigation, writing-original draft, visualization, data curation, formal analysis, project administration and fund acquisition. Sheikh Mustafizur Rahman and Shaikh Tareq Arafat Conceptualization, supervision, writing-review and editing, Md. Mostafizur Rahman and Md. Golam Sarower Writing-review and editing. Imran Noor, Iva Alam Pinkey and Zannatul Ferdoushe Investigation, data curation.

Conflict of Interest

The authors declare that they have no conflict of interest

Data Availability

Research data are not shared currently. However, the data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Approval

This work was carried out under the School of Life Science of Khulna University's Animal Ethics approval (KUAEC-2019/06/03).

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