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EFFECT OF TEMPERATURE FLUCTUATION ON THE INDIAN MAJOR CARP *LABEO ROHITA* AND *CIRRHINUS MRIGALA* LARVAE UNDER A CONTROLLED ENVIRONMENT

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ABSTRACT

Major carp i.e., Rohu (*Labeo rohita*) and Mrigala (*Cirrhinus mrigala*) are economically very important and commonly cultured in Indian subcontinent. In Pakistan aquatic organisms especially, freshwater species are facing many environmental problems such as variation in weather pattern. Temperature fluctuation is pervasive and most common environmental factor that is very distressing for major carps. The larvae of *Labeo rohita* and *Cirrhinus mrigala* were treated with different temperatures 10, 15, 20, 25, 30, 33 and 35 °C following 28 °C acclimation temperature. Thermal variation rate was recorded 2 °C/day. Death rate of both species larvae was observed. In temperature group 10 °C, 15% and 64% mortality of *Labeo rohita* was observed while, in *Cirrhinus mrigala* 16% and 66% mortality was recorded at 14 and 10 °C respectively. When larvae of both species were exposed to 10-20 °C, significantly ($P < 0.05$) maximum death rate was observed at this temperature as compared to other experimental groups. Cumulative death rate of *Labeo rohita* and *Cirrhinus mrigala* were recorded 84, 43, 22, 16, 1, 2, 3% and 86, 45, 23, 17, 1, 2, 4% in 10, 15, 20, 25, 30, 33, 35 °C temperature groups, respectively. All larvae of both species died within 2 days at temperature group 10 °C and continued to die at temperature groups 15 °C and 20 °C up to 11 days. Both species larval mortality persisted in temperature groups at 25 °C up to 18 days. Simple regression analysis was applied for thermal range ($T < 28$ °C and $T > 28$ °C), variation in death per fall and elevation of $\Delta T = 1$ °C was measured in the log-linear regression model framework. When water temperature dropped from acclimation temperature 28 °C, the accumulative death of larvae for 1 °C decrease of temperature from acclimation one, significantly ($P < 0.05$) was calculated in both species. When water temperature elevated from 28 °C, less steep beta coefficient was observed in *Labeo rohita* and *Cirrhinus mrigala*. 1 °C elevation of water temperature from acclimation temperature 28 °C, the accumulative death in *Labeo rohita* and *Cirrhinus mrigala* were ($P > 0.05$) obtained.

Keywords: Labeo rohita; *Cirrhinus mrigala*; Larvae mortality; Water temperature; Time series analysis

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INTRODUCTION

Fish have capability to confront with fluctuation of aquatic environment such as variation in weather pattern due to temperature fluctuation (Golovanova et al., 2013; Islam et al., 2022). Adaptation of freshwater fishes in thermal fluctuation reviewed to be a key instigation of their robustness, distribution, behaviour and partially controlled by their stress physiology (Alfonso et al., 2021; Chapman et al., 2022). Population of aquatic animals is declining worldwide due to climate change i.e. global warming (Fey and Greszkiewicz, 2021). Water temperature is most important and considered as a primary key to environmental factors regulating the growth performance, movement, disbursement and habits of freshwater fishes (Soriano et al., 2018; Yoon et al., 2022). Aquatic temperature has great influence on metabolism affecting the different physiological mechanism viz. feeding behaviour, swimming, reproduction and nutritional adaptability (Enders and Boisclair, 2016; Lindmark et al., 2022). Fluctuation in temperature above or below the thermal limit can persuade variation in immune system and provide direction for infectious disease (Cascarano et al., 2021; Marcogliese, 2008). Thermoregulation is an important mechanism of fish physiology and is most indispensable to expand their culture unit (Christensen et al., 2021; Hino et al., 2021).

In Pakistan, there is drastic fluctuation of environmental factors i.e., temperature, air, water and light throughout year (Dastagir et al., 2016; Hussain et al., 2022). During summer, highest temperature 50°C reaches in some areas of Baluchistan and Sindh (Ashraf et al., 2022). In winter temperature drops below -2°C in Skardu, Baltistan (Raza et al., 2015). Mostly teleostean species have peculiar adaptive mechanisms which skill them to confront against fluctuation in water temperature and enabled them to acclimatize in harsh domain (Abram et al., 2017; Blödorn et al., 2021). Variation in water temperature brings about increase energy demand and prompt increased glucose offering by balancing the carbohydrate metabolic pathway (Forgati et al., 2017; Jia et al., 2020). It is concluded that juvenile fish inhabiting at cooler region and have potential to adapt themselves in thermal circumstances, but this is ambiguous that the tropical populations have adequate acclimation ability to adjust further temperature increase (Eme et al., 2011; Pauly, 1984). The knowledge about behaviour of tropical fishes regarding the temperature fluctuation is confined therefore, it is rudimentary to investigate their response on temperature fluctuation (Figueira and Booth, 2010; Wilson et al., 2010). Drastic fluctuation in water temperature creates many complications for fishes and adversely affect their physiology such as air breathing, food consumption, digestive enzyme and reproduction (Schreck and Tort, 2016; Volkoff and Rønnestad, 2020). The food intake rate and enzyme efficiency reduced at temperature below 25°C (Ahmad et al., 2014; Guderley, 2004; Mandal et al., 2013).

Labeo rohita and *Cirrhinus mrigala* have very important economic, nutritional values and have geographic history (Panda et al., 2022; Ramachandran et al., 2008). These belong to family Cyprinidae, which is most dominant about 53.7%. Family Cyprinidae is most diverse has 84 species and 35 genera, and are the inhabitants of Ganga River channels in North India, Pakistan, Nepal and Burma (Sarma et al., 2017; Sheikh et al., 2017). Major carp breed in the river naturally (Rahman, 2008). Aquatic temperature played an important role in growth and development of major carps (Ashraf et al., 2022; Lavitra et al., 2010). These have wide range tolerance of temperature and salinity known as eurythermal species that flourish at water temperature 25-32 °C (Sharma et al., 2016).

There is limited knowledge regarding the impact of thermal variation on larvae stage *Labeo rohita* and *Cirrhinus mrigala* and effect of temperature fluctuation on physiological traits of fish larvae. Thermal variation is the powerful influencing pressure for poikilothermic organisms such as fish (Boltaña et al., 2017). The present study was designed to examined the impact of thermal fluctuation on *Labeo rohita* and *Cirrhinus mrigala* larvae and their survival rate, exposing to different temperature were recorded by using appropriate statistical analysis. This study manifests the adaptability of temperature-preference behavior and may be beneficial for predicting common features of temperature-preference correlation for the many species not yet scrutinized in this regard. This study could make it easier to comprehend how well these species can adapt to different temperatures.

MATERIALS AND METHODS

Ethical statement

This experimental study was conducted under the strict rules and regulation of Pakistan Animal Welfare Society. The fish larvae collection and managing were done under the instructions and guidelines of Fisheries Development Board (FDB). All possible efforts were made to minimize the pain and suffering of larvae during experiment.

Study area

This experiment was conducted in Aquaculture and Fisheries Laboratory, Department of Zoology, Wildlife & Fisheries, Pir Mehr Ali Shah Arid Agriculture University, at Rawalpindi, Punjab, Pakistan during month August 2022. This domain has temperature range of 1-42 ºC and having the average cloud cover 12 % (Khan et al., 2019; Waseem et al., 2022).

Experimental design

A total of 100 glass aquariums were selected for this experimental study and each aquarium masked with translucent fiber used as fish farming unit. They were washed with antiseptic potassium per magnate $KMnO_4$ prior to stocking (Ali et al., 2016). Each glass aquarium affixed with a purification/filtration section (Sera fil bioactive 130, Germany). Filtration portion minimized the nitrogen quantity in fish farming unit. For colling and heating system (HAILEA Chiller HC-300A, China and Sera Aquarium Heater 300, Germany) unit was connected with each aquarium to sustain the advisable temperature (Sharma et al., 2016).

Sample collection and acclimatization

Labeo rohita and *Cirrhinus mrigal* larvae were procured from Punjab, Fish, Seed Hatchery, Pakistan, having induced

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breeding resources. These species were acclimatized separately in outdoor cement tank at water temperature 28±2 °C. The weight of *Labeo rohita* and *Cirrhinus mrigala* larvae were measured (0.167±0.02 g) and (0.165±0.02 g) respectively, each species of major carp was randomly categorized in to seven groups and shifted into treatment groups. 50 fish larvae were introduced into each aquarium. The larvae of both species acclimatized for 7 days at water temperature 28 °C.

Larval feeding and maintenance of water quality parameters

Fish feed is composed of protein (40%) at the rate of 5 % during study duration. Larvae of both species fed diets by hand. Diet of both species was portioned into two sections and supplied once a day at 8.00 a.m. and 6.00 p.m. Physicochemical parameter of water were measured and checked by daily using multiple water quality measuring meter. Dissolve oxygen and pH of water were measured. pH value ranged from 7.50 to 8.03, 7.57 to 8.15, 7.87 to 8.40, 7.70 to 8.60, 7.70 to 8.44, 7.34 to 8.50, 7.40 to 8.60, and dissolved oxygen ranged from 5.55 to 6.10, 5.30 to 6.20, 5.50 to 6.40, 5.55 to 6.20, 5.10 to 6.30, 5.12 to 6.10, 5.03 to 6.14 mg/l at 10, 15, 20, 25, 30, 33 and 35 °C of experimental groups, respectively during the study period.

Temperature range

Larvae of both species were exposed to 7 different temperatures range 10, 15, 20, 25, 30, and 35 °C and were maintained simultaneously under laboratory environment (Table 1). Control groups of both species were held in outdoor environment. Three replicates of each species were operated for each treatment. Each treatment acquired at the rate of change of 1°C per 12 h (2 °C/day) beginning from acclimation temperature. Desirable temperature obtained within 1-9 days. Temperatures range was maintained during experimental study because this temperature is predominating in different domains of Pakistan.

Table 1. Planned and measured water temperature (±SE) in each treatment of *Labeo rohita* and *Cirrhinus mrigala* larvae during experiment.

Treatments (°C)	Planned temperature (°C)	Measured temperature of *Labeo rohita* larvae (°C)	Measured temperature of *Cirrhinus mrigala* larvae (°C)
10	10	10.10±0.091	10.13±0.095
15	15	15.17±0.085	15.15±0.096
20	20	20.32±0.165	20.12±0.95
25	25	25.27±0.138	25.27±0.111
30	30	30.40±0.108	30.53±0.149
33	33	33.35±0.155	33.42±0.155
35	35	35.15±0.155	35.20±0.158

Statistical analysis

Mortality of fishes

Due to variation in temperature from acclimation point larvae of both species showed mortality in experimental group. Expired/dead fishes of experimental groups were amassed regularly from each treatment and counted. Experimental data of both species were arranged as standard error mean. Data of both species were analyzed by applying suitable statistical analysis one-way analysis of variance (ANOVA). Significantly ($P < 0.05$) was accepted (Sharma et al., 2016).

Time Series analysis

Simple linear regression of cumulative mortality was applied against the temperature range to determine the slope factor, or percentage changes of mortality per 1°C variation of temperature:

$$Ln(M) = Const. + \beta\ (T_{max} - T_{min}) + \varepsilon$$

Where, *Ln* (*M*) = Log- transformed mortality, β = slope factor, T_{max} and T_{min} = maximum and minimum temperature, respectively and ε = residual term

Increase in temperature meaning raising temperature from 28 °C, while decrease in temperature implied reducing temperature from acclimation one. To obtained a normal distribution of the dependent variable, log-transformed mortality was applied. By using regression equation, experimental temperature for both species *Labeo rohita* and *Cirrhinus mrigala* lies between 28 and 25 °C, 28 and 20 °C, 28 and 15 °C, 28 and 10 °C and above acclimation, temperature range 28-30 °C, 28-33 °C and 28-35 °C were recorded individually (Table 2).

RESULTS AND DISCUSSION

The water temperature of different experimental groups 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, 33 °C and 35 °C of *Labeo rohita* and *Cirrhinus mrigala* gradually decreased or increased from acclimation temperature 28 °C, induces the mortality of larvae. In temperature group 10 °C, as a temperature reached 16 °C, mortality of *Labeo rohita* and *Cirrhinus mrigala* larvae started, 15% and 16% of mortality were observed at 14 °C, 32% and 33% larvae of both species died suddenly as a water temperature reached 10 °C respectively. All larvae of *Labeo rohita* (64%) and *Cirrhinus mrigala* (66%) mortality was observed within two days after reaching 10 °C temperature. In temperature group 15 °C, 4% and 5% death rate were observed in *Labeo rohita* and *Cirrhinus mrigala* at water temperature range 24-18 °C respectively. Further water temperature started to decrease from 15 °C, *Labeo rohita* (16%) and *Cirrhinus mrigala* (17%) mortality of larvae was observed. The death rate of both species was recorded and death continued up to 11 days. 3% death rate was observed in *Labeo rohita* in experimental group 20 °C while, in *Cirrhinus mrigala* it was 4%. 7% and 8% mortality rate were observed in *Labeo rohita* and *Cirrhinus mrigala* due to sudden drop in temperature from 24 °C to 20 °C and continued up to 11 days **(Figure 1) and (Figure 2).** 2% mortality was recorded in both species within 4 days at 25 °C treatment group and death remained continue in both species up to 18 days. 1% mortality of larvae was recorded in both species within 10 days at experimental group 30 °C and 2% death rate was observed in both species within 24 hours at 33 °C experimental group. Further 1% and 2% death rate were observed in *Labeo rohita* and *Cirrhinus mrigala* within 2 days and 2% mortality was recorded in both species on day-3 at experimental group 35 °C (Table 2). In control group both species showed no mortality during the study duration.

Table 2. Mortality of *Labeo rohita* and *Cirrhinus mrigala* versus range of water temperature.

Temperature range	Difference	Mortality rate of *Labeo rohita* (%)	Mortality rate of *Cirrhinus mrigala* (%)
T < 28 °C			
28-25 °C	3	16	17
28-20 °C	8	22	23
28-15 °C	13	43	45
28-10 °C	18	84	86
T > 28 °C			
28-30 °C	2	1	1
28-33 °C	5	2	2
28-35 °C	7	3	4

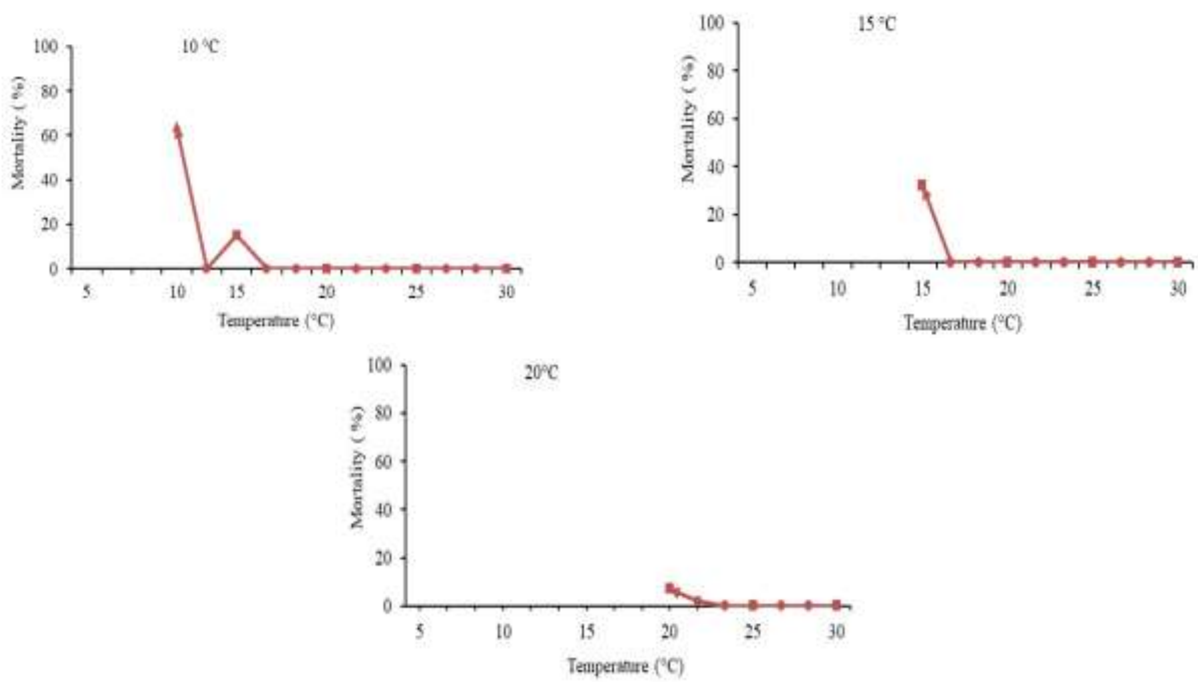

Figure 1. Mortality of *Labeo rohita* exposed to temperature 10, 15 and 20 °C.

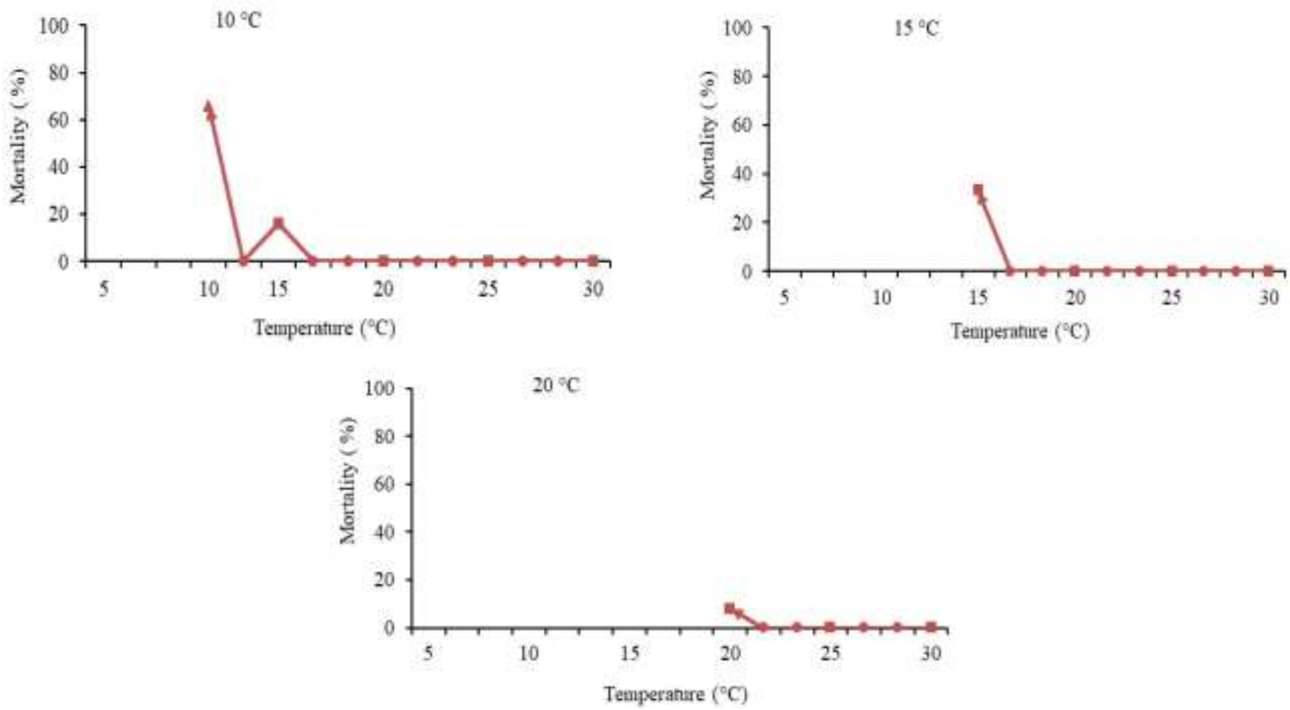

Figure 2. Mortality of *Cirrhinus mrigala* exposed to temperature 10, 15 and 20 °C.

Among all these experimental groups, *Labeo rohita* and *Cirrhinus mrigala* showed higher accumulative mortality ($P < 0.05$) was calculated at lowest temperature 10 °C as compared to other experimental groups. Cumulative mortality rates of *Labeo rohita* and *Cirrhinus mrigala* were recorded 84, 43, 22, 16, 1, 2, 3% and 86, 45, 23, 17,1 ,2, 4% at 10, 15, 20, 25, 30, 33, 35 °C treatment groups, respectively **(Figure 3) and (Figure 4).** When *Labeo rohita* and *Cirrhinus mrigala* larvae were treated above acclimation temperature 1-3% and 1, 2, 4% mortality were recorded.

Statistical approach indicated that there was no significant difference ($P > 0.05$) among these experimental groups was calculated, when temperature rises above acclimation point. This experimental study showed that decrease of temperature from acclimation point 28 °C, increases the physiological disturbance and more stressful for *Labeo rohita* and *Cirrhinus mrigala* as compared to upraising of temperature from acclimation point. The capability of freshwater fishes to tolerate thermal stress depends upon the acclimation temperature (Zhu et al., 2022). 7 °C fluctuation of water temperature in both species *Labeo rohita* and *Cirrhinus mrigala* was recorded between highest temperature 35 °C and the acclimation temperature 28 °C. There was 18 °C variations of water temperature in both species were calculated between lowest temperature 10 °C at which larvae of both species were exposed and acclimation temperature 28 °C. The highest temperature, 35 °C treatment group in current study was 3 °C higher as compared to optimum range of 25-32 °C (Das et al., 2004; Day, 2014). The Current study indicated that at 10 °C all larvae of both species *Labeo rohita* and *Cirrhinus mrigala* expired within 2 days. Mortality of both species remained continue up to 11 days at 15 and 20 °C experimental temperature. In India researcher conduct a similar study to determine the impact of water temperature on adult *Clarias batrachus* exposed to water temperature range 10-35 °C. Statistical analysis showed that higher mortality rate was observed at 10 °C, significantly ($P < 0.05$) mortality of fish was calculated at lowest temperature 10 °C as compared to other experimental groups. 50 % fish mortality was recorded within 5 days at temperature group 10 °C (Singh et al., 2013).

In India researcher conducted a study on fish *Catla catla* larvae to examined the impact of temperature on fish larvae by exposing them with temperature range 10-35 °C, which supports the current findings. Accumulative death rates of *Catla catla* was found 89, 43, 24, 18, 1, 2 and 3% at temperature groups 10, 15, 20, 25, 30, 33, and 35 °C respectively. Statistical record showed that significantly ($P < 0.05$) higher death rate was observed in larvae a water temperature range 10-20 °C as compared to other experimental groups (Sharma et al., 2016). Temperature has

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great thermodynamics impact on fish biochemical reactions rates (Killen, 2014; Little et al., 2020). Lower water temperature has negative effect on fish cellular and hormonal response (Herbing, 2002; Lindmark et al., 2022).

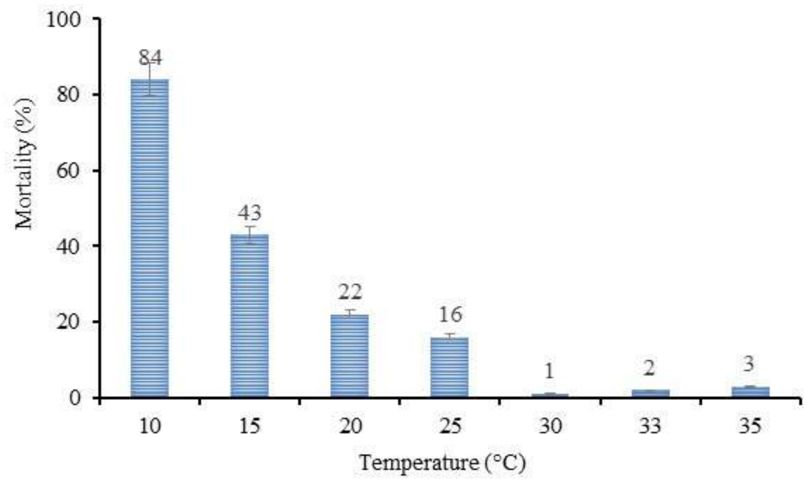

Figure 3. Cumulative mortality of *Labeo rohita* exposed to various temperature.

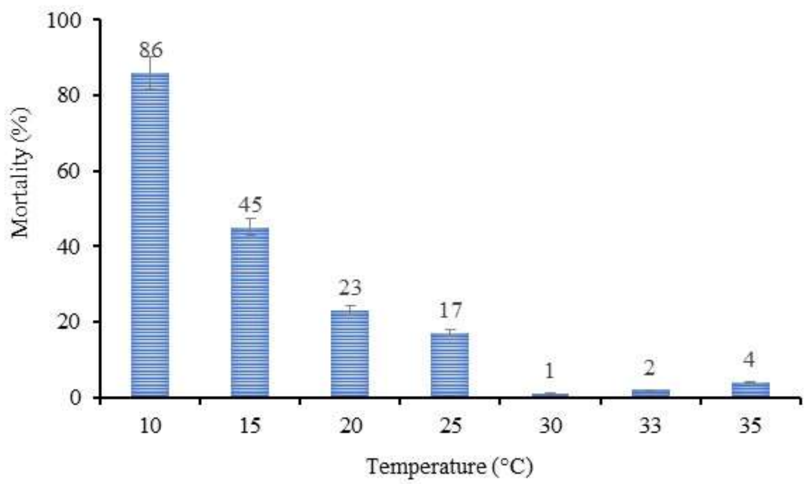

Figure 4. Cumulative mortality of *Cirrhinus mrigala* exposed to various temperature.

Fluctuation of water temperature range from acclimation point (T < 28 °C and T > 28 °C was evaluated by using simple regression analysis. Variation in death rate of *Labeo rohita* and *Cirrhinus mrigala* due to decrease or increase of temperature (ΔT =1°C) was measured by applying framework of log-linear regression model. This model indicates that when water temperature started to drop from acclimation point 28 °C, significantly ($P < 0.05$) accumulative death rate for both species *Labeo rohita* and *Cirrhinus mrigala* was calculated. When water temperature started to accelerate above acclimation point 28 °C, less steep beta coefficient was observed. Significantly, ($P > 0.05$) the accumulative death for both species was observed due to 1°C increase of water temperature from acclimation temperature 28 °C. In temperate and cold domains, temperature-mortality increases as water temperature fall below or uprising above the acclimation temperature (Sharma et al., 2016).

CONCLUSION

This experimental study concluded that lowering of water temperature 15 °C from acclimation temperature 28 °C was distressing, and stressful to the larvae of *Labeo rohita* and *Cirrhinus mrigala* resulting mortality occurred. Temperature reductions of one degree from acclimation temperature caused death of *Labeo rohita* and *Cirrhinus* *mrigala* 10.5 % and 10.6 % respectively. Both are tropical species, showed adaptability at high temperature. Water temperature is most important for ensuring survival and very affecting for growth, development and reproduction. The inability of fishes physiologically adopt to temperature fluctuation is typically attributed to their mortality.

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CONFLICTS OF INTEREST

The authors declared no conflict of interest. The funders had no part in the design, collection analyses and interpretation and writing of short communication.

AUTHOR'S CONTRIBUTION

Muhammad Shahbaz Azhar: Execute the experiment, Data curation, Visualization, Investigation, Writing – original draft. Muhammad Zubair Anjum: Supervision, Review and editing. Shamim Akhter: Conceptualization, Resources. Muhammad Qayash Khan: Writing- review and approved the final manuscript. Shaista Bibi: Supervision, Investigation, Fatima Rasool: Statistical analysis

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