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Effect of Solar Radiation on Freshwater Fish *Puntius bimaculatus* (Bleeker) (Cypriniformes: Cyprinidae)

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Abstract: Environmental and physical stressors such as sunlight, atmospheric temperature, photoperiod, and UV radiation have a significant impact on all living organisms in the aquatic ecosystem, particularly on rocky shores, shallow water bodies, lakes, and ponds. In this study, the freshwater fish *Punitus bimaculatus* (Bleeker) (Cypriniformes: Cyprinidae) was exposed to solar radiation for 1h to 2h duration during the summer season from March to May (water temperature 40 ºC to 42 ºC). The stress caused by increased water temperature (40 °C to 42 °C) significantly reduced the physiological activities and alter the metabolism of the fish, particularly carbohydrate, protein, and lipid levels in the experimental fish, and also damaged the muscle tissue. The muscle carbohydrate, protein and lipid content of *Punitus bimaculatus* reduced when exposed at 40 ºC and 42 ºC . This could be due to the stress-induced on the fish to utilize the stored energy in the body. *Punitus bimaculatus* muscle tissue showed marked degeneration of the epithelium, disappearance of striations, and thickened septal wall. The continual degradation of primary metabolites is vital for tissue maintenance but is also important in allowing animals to adapt to changing environmental conditions, replace denatured or damaged proteins, mobilize amino acids, and allow metabolic regulation.

Keywords: Stress response, Water quality, Temperature, Biochemistry, Histology, Global climate change

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Introduction

The global temperature has been rising in recent years, owing primarily to anthropogenic activities (Häder *et al.*, 2015). Indiscriminate use and discharge of industrial effluents, air pollutants, CFCs, and greenhouse gases are the main reason for increasing temperatures. It directly affects all ecosystems. Aquatic organisms are often exposed to a number of stressors when they live in an

environment that has been misused by human beings. Most living organisms frequently respond to changes in their environments through different physiological mechanisms in natural habitats (Louiz *et al.*, 2018)

Solar radiation, photoperiods, and temperature have been shown to significantly harm living organisms such as amphipods, isopods, mollusks, fishes, eggs, and larva of many organisms in the aquatic ecosystem, particularly rocky shores, shallow water bodies, lakes, and ponds. They exhibit a variety of symptoms, including behavioral, cytological, genetical, biochemical, and physiological changes (Li *et al.*, 2011; de Ramos *et al.*, 2019; García-Garay *et al.*, 2020; Lawrence *et al.*, 2020). Biochemical indicators, particularly the total protein, carbohydrate and lipid level of the organisms, are useful for the assessment of stress and environmental changes or modifications if any in the ecosystem (Chang, 2005; Häder *et al.*, 2015; Hook *et al.*, 2018; Rosic *et al.*, 2020; Duan *et al.*, 2021).Temperature affects the muscle tissues (epidermis, and dermis), total protein, carbohydrates, and lipid (Ong *et al.*, 2018). The impact of solar radiation and temperature on fish is very scanty (Vargas *et al.*, 2018). There is an urgent need to study the ongoing global warming issues in all ecosystems.

In this study, the freshwater fish *Punitus bimaculatus* (Bleeker) (Cypriniformes: Cyprinidae) was exposed to solar radiation for 1h to 2h duration during the summer season from March to May (water temperature 40 ℃ to 42 ℃). The carbohydrate, protein and lipid content of muscle and histological changes in muscles were examined after exposure to solar radiation.

Materials and Methods

Sample collection:

Healthy living specimens of *Punitus bimaculatus* (7.0 g; 5cm) were collected from Thandavarayankulam Pond, Srivaikuntam Taluk, Thoothukudi District, Tamil Nadu, india. They were transported to the laboratory and maintained in a fresh water fiber tank (1000 L). This experiment was carried out in accordance with Department of Animal Science, M.S. University, Tirunelveli guidelines for the care of the experimental fishes. The safety and ethical protocol were followed during the experiments and Asphyxia or mortality of fish were not encountered during this experiment. The fish were maintained for acclimatisation in a well-aerated fresh water fish tank with the following parameters: 1000 L capacity; pH 7.4; water temperature of 26 °C; photoperiod 12:12 (light:dark).

Experimental set up:

In this study, the control fish *Punitus bimaculatus* (n=30) was kept inside the fish tank (fiber tank 1000 L). The lethal dose of water temperature and solar radiation on *P. bimaculatus* was recorded for 10 days during the summer season from March to May. After that, a standardized protocol was followed for this study. In the experiment, *P. bimaculatus* were introduced into the fresh water, which is heated under direct sunlight at 11 AM. *Punitus bimaculatus* was transferred to a plastic bucket with a total capacity of 15 liters, of which 12 liters were filled with water. The fresh water in a plastic bucket was placed in the sunlight at 8 a.m., and the water and atmospheric temperatures were recorded at that time. When the temperature reached 40 °C, fish (n=6) were introduced into the plastic bucket. Three fish were taken from the experimental bucket after 1 h and labeled as 1 Hour Temperature Inoculated fish (1HTIF) 40 ℃ (water temperature). After 2 h, three fish were removed from the experimental bucket and labeled as 2 Hour Temperature Inoculated fish (2HTIF) 42 ℃ (water temperature). Then the experimental and control *P. bimaculatus* were dissected and muscles were extirpated for histological and biochemical analysis.

Histology and Biochemical analysis:

The histological observations of control, 1 Hour Temperature Inoculated fish (1HTIF), and 2 Hour Temperature Inoculated fish (2HTIF) solar

Table 1: Biochemical changes in muscle tissue of freshwater fish *P. bimaculatus* exposed to solar radiation (temperature) at 40 °C and 42° C

Tissue tested	Biochemical constituents	Control (mg/100 mg wet tissue)	Duration of exposure* (mg/100 mg wet tissue)	
			1 HTIF	2 HTIF
Muscle	Total Protein	2.122	1.221	1.007
	carbohydrate	1.311	0.144	0.102
	lipid	0.196	0.167	0.033

Values are expressed mean; HTIF Hour Temperature Inoculated Fish

radiation-exposed freshwater fish *P. bimaculatus* muscle tissue were processed to see if there were any changes in muscle tissue. The muscle tissue samples of *P. bimaculatus* were sliced and fixed using 50% formaldehyde for 24 h. Then the tissues were gently washed with distilled water. After routine paraffin embedding sections were cut at 6 µm and stained with Hematoxylin and Eosin. The slides were observed under a phase contrast microscope (Subramanian *et al.*, 2017). The control and experimental (40 ℃) 1 Hour Temperature Inoculated fish (1HTIF) and (42 ºC) 2 Hour Temperature Inoculated fish (2HTIF) Solar radiation exposed fish *P. bimaculatus* muscle tissue were taken for biochemical analysis such as protein (Lowry *et al.*, 1951), carbohydrate (Roe, 1955), and lipid (Folch *et al.*, 1957).

Results and Discussion

The carbohydrate, protein, and lipid content in muscle tissue were analyzed in the freshwater fish *Punitus bimaculatus* at room temperature (control), exposed at 40 ℃ (1HTIF) and 42 ℃ (2HTIF). The results are summarized in Table 1. The muscle carbohydrate content of *Punitus bimaculatus* reduced from 1.311 mg/100 mg wet tissue (control) to 0.144 mg/100 mg wet tissue when exposed at 40 ℃ (1HTIF), and further reduced to 0.102 mg/100 mg wet tissue in 2HIFT exposed individuals. The protein content in the muscle tissue of *Punitus bimaculatus* showed a decreasing trend with increasing time of exposure (solar radiation). The total protein content in the muscle tissue of control *Punitus bimaculatus* was 2.122 mg/100 mg wet tissue and it reduced gradually to 1.221 mg/100 mg wet tissue (1HTIF) and 1.007 mg/100 mg wet tissue in 1HTIF and 2HTIF, respectively. Likewise, the muscle total lipid content showed gradual reduction with increasing time of exposure. In control, the lipid level was 0.196 mg/100 mg wet tissue. Muscle lipid levels in 1 h and 2 h exposed fishes were 0.167 mg/100 mg wet tissue and 0.033 mg/100 mg wet tissue, respectively.

The histological structure of muscle of control *P. bimaculatus* has been shown in Figure 1. The muscle of experimental fish *P. bimaculatus* exposed to 40 ℃ (Fig. 2) and 42 ℃ (Fig. 3) showed marked degeneration of the epithelium, disappearance of striations, thickened septal wall, broken fiber.

In the present investigation, the temperature has induced alternations in the biochemical constituents such as protein, carbohydrates and lipid. The decrease in the protein content was much more pronounced in the muscle tissues at 40º C during 1 h duration. The protein level showed a decreasing trend in the muscle tissues at longer exposure period (2 h) at 42 ℃. The continual synthesis and degradation of protein is not only vital for tissue maintenance and animal growth but is also important in allowing animals to adapt to changing environmental conditions, to replace denatured or damaged proteins, to mobilize amino acids and to allow metabolic regulation (Hawkins and Hilbish, 1992).

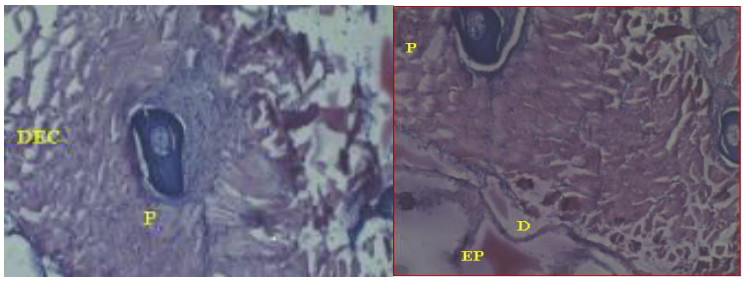

Fig.1: Photomicrographs of control fish *P. bimaculatus*. Haematoxylin and eosin. DEC- Dence Epidermal Cells, P –Pigment Cells, EP-Epidermis

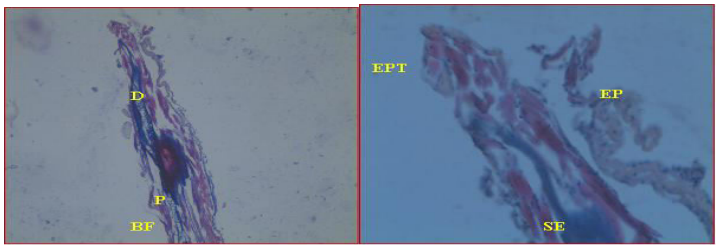

Fig. 3: Photomicrographs of muscle of fish *P. bimaculatus* exposed to solar radiation (temperature) at 40 °C muscle. Haematoxylin and eosin. BF- Broken Fibre, SE- Squamous Epithelium, D- Dermis, P –Pigment Cells, EP-Epidermis, EPT- Epithelial Tissue.

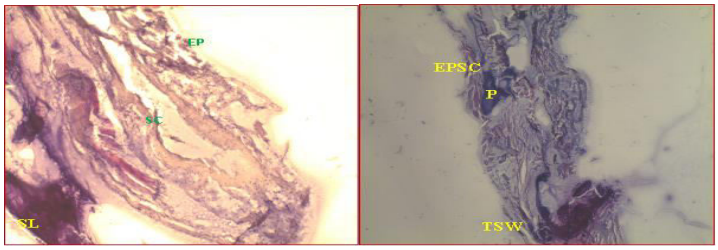

Fig. 4: Photomicrographs of muscle of fish *P. bimaculatus* exposed to solar radiation (temperature) at 42° C. Haematoxylin and eosin. TSW- Thickened Septal Wall, SE- Squamous Epithelium, P –Pigment Cells, Ep-Epidermis, SL- Stratum Laxum, SC-Stratum Compactum

Water is the major part of the crust; the aquatic ecosystem encounters enormous adverse effects (Häder *et al.*, 2015). Because fishes are such an important part of both freshwater and marine ecosystems, there is a lot of interest in studying them to improve diversity. Generally, during unfavourable or stress conditions, fishes require extra energy to restore the normal physiological and biochemical changes for better survival. This excess energy needed may be obtained by oxidizing the body's reserves such as carbohydrates, protein, and lipids (Ganesan *et al.*, 1989). Temperature significantly influences the ability of the fish to collect or capture the food, digestion and absorption of nutrients. The short term changes in water temperature affect the fish physiology (Volkoff and Rønnestad, 2020).

This study clearly demonstrated that raising the water temperature by 1 ℃ for 1 h duration, alter the physiology of the experimental fish. If the water temperature is raised above 42 ℃, there is possibility of mortality of experimental fish *P. bimaculatus*.

Conclusion

This present study was performed on the effect of raised temperature (40 ℃ and 42 ℃) on biochemical and histopathological changes in muscles of fresh water fish *P. bimaculatus*. The marked variation in the biochemical parameters and changes in muscle tissue were observed. Protein, carbohydrates, and lipid contents of

muscles were reduced at 40 ºC and 42 ºC as compared to the control fish. The muscles showed degeneration of the epithelium, disappearance of striations, thickened septal wall and broken fiber.

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