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IMPACT OF TEMPERATURE FLUCTUATIONS ON GUT HISTOLOGY OF THE CRICKET FROG, *FEJERVARY ALIMNOCHARIS* (ANURA: DICROGLOSSIDAE)

M M Rahman*

Department of Zoology, Jahangirnagar University, Savar, Bangladesh

Abstract

Context: Research work was conducted on Impact of temperature fluctuations on gut histology of the cricket frog, *Fejervary limnocharis* (Anura: Dicroglossidae).

Objectives: To understand relationship between histological structure and physiological activities as per periodic life styles of the cricket frog.

Materials and methods: The specimens were collected from different parts of Bangladesh. The basic structure of gut of this frog species were comprised of four layers, viz., serosa, muscularis mucosa, sub-mucosa and mucosa in general.

Results: The study revealed that the temperature has immense effect on gut structure of *F. limnocharis*. Muscularis mucosa was wanting in esophagus whereas serosa was the thinnest layer in stomach and intestine in low temperature, 0.02 mm ±0.01 and 0.01 mm ±0 respectively. Astonishingly muscularis mucosa was measured as the thickest layer in esophagus in high temperature, 3.2 mm ±0.2, instead of mucosa, 3.1 mm ±0.152753, which was the thickest layer in stomach and intestine, 2.6 mm ±0.2 and 1.9 mm ±0.450925 respectively. All the layers of gut responded positively to the raised temperature. From this study it can be concluded that temperature fluctuation followed by changes in physiological activities, as in hibernation, may influence the histological structure.

Key words: High temperature, low temperature, cricket frog, digestive tract.

Introduction

Amphibian and reptilians are very sensitive to vicissitudes in environmental temperature, precipitation, and the hydro period (Carey and Alexander 2003). Amphibian and reptilians may show irregularities in calling, breeding, egg laying, metamorphosis, dispersal and migration in response to changes in rainfall and temperature fluctuation (Beebee 1995). Growth and survival rate may also be affected if such budges occur inconsistently with other ecological events e.g., emergence of their prey species (Lind 2008). Being cold blooded animal, body temperature and activity cycles of amphibians are strongly dependent on environmental conditions. Beside this, in many cases amphibians need aquatic habitats for egg laying and larval development and even for post-metamorphic life stages they require at least moist conditions (Duellman and Trueb 1986, Wells 2007).

The amphibians are unavoidable constituents of most ecosystems and even in some cases they make maximum part of the vertebrate biomass (Burton and Likens 1975). Having diversified mode in their life cycle, aquatic immature and terrestrial mature form, they are important in nutrient cycling between different environments (Storer *et al.* 1972). Eating prey species like insects and being eaten by predators like birds and mammals, they play important role in flowing energy in the ecosystems (Blaustein and Wake 1990). Amphibians are often referred as Farmer's Friend, as they mostly feed on small invertebrates inhabiting in crop fields as pests (Dutta and Mohanty-Hejmadi 1978). Moreover, they are considered as useful environmental indicators (Sheridan and Olson 2003, Hammer *et al.* 2004, Collins and Storfer 2003). This species was selected for its abundance and availability (Rahman *et al.* 2013) at everywhere of the study area

* Corresponding author *E-mail:* mizanur32ju@gmail.com

and its conspicuous character (Rahman *et al.* 2012) which made easier to catch them. Though some studies on histology, physiology and metabolic rate of American cricket frog (Irwin *et al*. 1999, Swanson and Burdick 2010) and other amphibians (Chen and Liu 2012, Jameson *et al.* 1970) have been conducted but there is no such work on any species bring into being in Bangladesh yet and this lead to the present study. The main aim of this study was to find out the probable changes in the gut structures following alteration in physiological activities due to temperature fluctuations.

Methods and Materials

In order to understand the impact of temperature fluctuation on gut of *F. limnocharis*, its histology of esophagus, stomach and intestine were investigated. The structure of these organs was observed in low and high temperature periods. The specimens were collected from the places having more or less homogeneous conditions. It is believed that the food sources were more or less equally available in that area throughout the study period. However, 12◦C to 20◦C (November-February) was determined as low temperature and 21◦C to 35◦C (March-October) as high temperature period. The specimens were collected from low, marshy areas of different parts of Bangladesh (Rangpur, Maulvi Bazar and Dhaka district). Immediately after collection, time, site of the collection, habitat type etc. of each sample was recorded and the specimens were transported in live condition to the research laboratory, Department of Zoology, Jahangirnagar University, Savar, Bangladesh. A total of 78 individuals were collected, 42 in high temperature period and 36 in low temperature period, to conduct the present study.

The collected individuals were identified through matching the characteristics described and photographs given in different books, websites, journals and magazines. Simultaneously, help from the experts was also taken in order to identify the individuals.

To prepare the histological slides, first of all, the specimens were dissected and their esophagus, stomach and intestine were collected. Then the organs were washed with normal saline water and kept in Buin's fluid for 12 hours. After that these were treated with 70% alcohol and distilled water and then cut into small square sized pieces and allowed for dehydration by successively graded ethanol mixture. The pieces were then dealcoholized, impregnated, embedded and made into small blocks using xylene, melted wax and glycerin. Then the blocks were cooled and trimmed to bring the tissue at the surface. Serial sections were cut with the help of a microtome machine at 5 to 6 micron. The tissue ribbons were then affixed and stretched on a slide using Mayer's albumen. After treating with xylene these slides were transferred to descending graded alcohol as the tissues can be stained by hematoxylene and eosin. Stained slides were then passed through ascending graded alcohol, cleaned with xylene and mounted applying Canada balsam and placing a cover slip on the tissue. Finally, the prepared slides were examined and studied by a compound microscope in the laboratory. The microphotography was done by using a microscope (Olympus CH30/CH40, Japan) and a digital camera.

Acquired data from present study were statistically analyzed through Student's t-test.

Results

The histological structure of gut of *F. limnocharis* changes with the temperature fluctuation followed by changes in physiological activities. In most cases the structure of organs become emaciated and loses some of their ordinary layers and sub-layers in low temperature and distended in high temperature. However, all parts of digestive tract of the subject species were mainly made up of four layers, such as, serosa, muscularis mucosa, sub-mucosa and mucosa.

In low temperature esophagus consisted of three layers, a muscularis mucosa layer was wanting, while other organs, stomach and intestine, had all the four ordinary layers. Mucosa was the thickest layer in esophagus, muscularis mucosa in stomach although both sub-mucosa and mucosa had the same supreme reading in intestine in low temperature. Serosa was the thinnest layer all over the digestive tract in this temperature frontier (Table1, Fig. 1).

Table 1. Measurement of different layers of digestive tract, esophagus, stomach and intestine, of *Fejervary limnocharis* in low temperature.

Organs / Layers	Esophagus (mm)	Stomach (mm)	Intestine (mm)
Serosa	1.1 ±0.208	0.02 ±0.01	0.01 ±0
Mucularis mucosa	0 ±0	0.5 ±0.173	0.05 ±0.02
Sub-mucosa	1.4 ±0.2	0.08 ±0.01	1 ±0.1
Mucosa	1.8 ±0.1	0.4 ±0.1	1 ±0.173

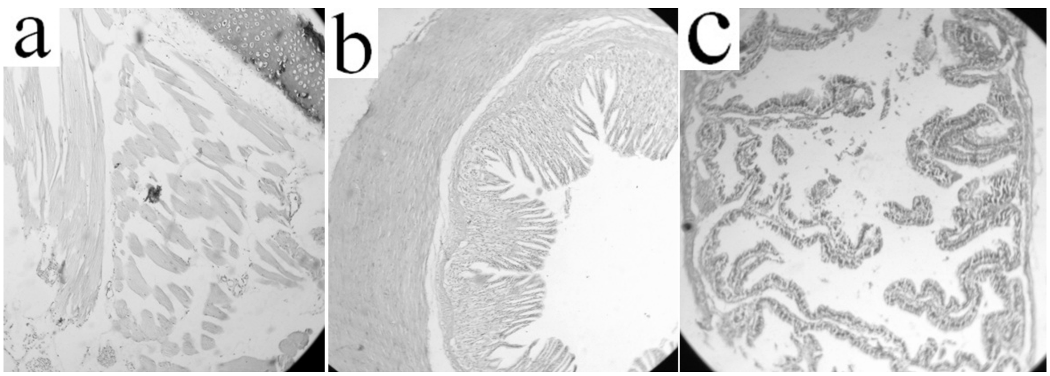

Fig. 1. Microscopic view of different layers of **a.** esophagus, **b.** stomach and **c**. intestine of *F. limnocharis* at low temperature.

In high temperature esophagus regained muscularis mucosa layer and hence entire digestive tract of cricket frog was made up of all the ordinary four layers in this period. Musclaris mucosa was the thickest layer in esophagus while mucosa in stomach and intestine in high temperature. Serosa was the thinnest layer in esophagus and intestine whereas sub-mucosa in stomach (Table 2, Fig. 2).

Table 2. Measurement of different layers of digestive tract, esophagus, stomach and intestine, of *Fejervary limnocharis* in high temperature.

Organs / Layers	Esophagus (mm)	Stomach (mm)	Intestine (mm)
Serosa	1.1 ±0.173	2.5 ±0.264	0.07 ±0.020
Mucularis mucosa	3.2 ±0.2	2.2 ±0.2	1 ±0.2
Sub-mucosa	1.8 ±0.1	0.9 ±0.1	1 ±0.2
Mucosa	3.1 ±0.152	2.6 ±0.2	1.9 ±0.450

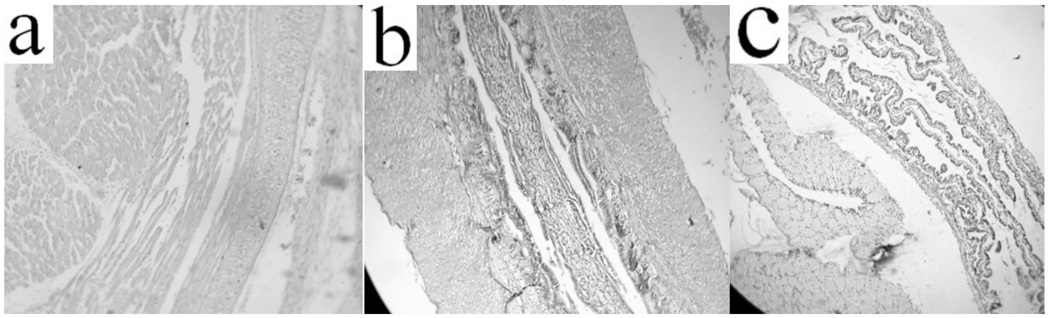

Fig. 2 Microscopic view of different layers of **a.** esophagus, **b.** stomach and **c.** intestine of *Fejervary limnocharis* in high temperature.

The observed data were tested through Student's t-test and found almost all layers of different parts of digestive tract showed significant differences in low and high temperature in cricket frog. However, serosa of esophagus and sub-mucosa of intestine had insignificant differences in these periods. Muscularis mucosa (t=27.83) and mucosa (t=12.96) of esophagus and serosa (t=16.21), sub-mucosa (t=12.41) and mucosa (t=17.054) of stomach were significant at 0.1% level. On the other hand, muscularis mucosa (t=7.025) of stomach and serosa (t=4.75) and muscularis mucosa (t=8.19) of intestine were significant at 1% level while sub-mucosa (t=3.1) of esophagus and mucosa (t=3.32) of intestine were significant at 5% level.

Discussion

Like other species of *Fejervarya*(Chen and Liu 2012), present study also found four layers of alimentary canal, viz, serosa, muscularis mucosa, sub-mucosa and mucosa. The present study shows that many of these layers of observed organs were absent or at least thinned than the normal in low temperature (hibernation) period. This may happen due to lessening metabolic activities (Jameson *et al*. 1970, Tashian and Ray 1957, Dunlap 1972) and reducing heat loss to the surroundings (Bullock 1955, Prosser 1964). Being ectotherms, both of these actions are very important for amphibians to cope with low temperature period (Geiser -Fritz 2008). They generally stop feeding, while their prey species (Khan 2008) go to diapause and this can be another possible reason behind this (Lind 2008, Shinde 2013, Su *et al.* 1997, Shi and Ishuzuya-Oka 1996). On the other hand, like many other frogs which show correlation between size and activity of corresponding organs (McClelland *et. al.* 1996), the structures became well developed even with more layers on rising temperatures. It suggests that the increased temperature enhance the physiological activities, especially metabolic rate, consequently development of internal organs and growth rate of the individual. This phenomenon is somewhat controversial with many researches (McMenamina *et al.* 2008, Reading 2007) but supported by a number of other studies (Kuan and Lin 2011, Banks and Beebee 1988) Kuan and Lin (2011) had very specific observations for *F. limnocharis.* They showed that both summer and spring cohort of *F. limnocharis* tadpoles responded to high temperature with accelerated developmental rates and conclude with 'higher growth rate of tadpoles of Indian cricket frog indicates the increased temperature of a particular area'.

Conclusion

Though this study has not completely unveiled the obscurity, it will help upcoming researchers to understand the amphibian histology, mystery of their hibernation, and to some extent, about how they will cope with temperature fluctuations caused by climate change. From the above scenario it can be concluded that gut structures of *Fejervary limnocharis* respond positively to increased temperature together with physiological activities.

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