

Histological Study of Bullfrog (*Hoplobatrachus Tigerinus*) Organs Exposed to Habitat Stress

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ABSTRACT

Hoplobatrachus tigerinus, commonly known as the Indian bullfrog, is a large amphibian belonging to the family Dicroglossidae. It is widely distributed in South Asian countries such as India, Pakistan, Nepal and Bangladesh. This species usually inhabits freshwater environments. The frog possesses strong hind limbs that help in jumping and swimming, and it mainly feeds on insects and small invertebrates. Due to its sensitivity to environmental changes and pollutants, *Hoplobatrachus tigerinus* is often considered an important organism in ecological and environmental research. The present study was carried out on *Hoplobatrachus tigerinus* (bullfrog) to examine the histological structure of internal organs such as lungs and kidneys exposed to habitat stress and to analyze the occurrence of melanophores in relation to environmental conditions. Amphibians are considered important ecological indicators due to their permeable skin and sensitivity to environmental changes. Amphibians play an important role in maintaining ecosystem balance and they highly respond to ecological stress such as pollution (Bunchaen et al., 2022). The lungs of *H. tigerinus* are thin-walled sac-like respiratory organs with internal folds that increase the surface area for efficient gaseous exchange and has multiple layers including mesothelium, connective tissue, smooth muscle, and respiratory epithelium (Hermida et al., 1998). The kidney of the *H. tigerinus* consists of numerous nephrons which are the structural and functional units of the kidney. Each nephron is composed of a Bowman's capsule, glomerulus, renal tubule and lumen. These structures work together to filter blood, remove waste products, and regulate water and salt balance in the body (Rant and Stewart, 1943). The melanophores present in amphibian skin act as pigment cells and they are also seen in vital organs when they are exposed to the environmental stress, ultraviolet radiation and temperature fluctuation (Pshennikova and Voronina, 2018). Therefore, variations in melanophore distribution may serve as an indicator of environmental pollution.

Keywords: *Hoplobatrachus tigerinus*, lungs, kidney, melanophores, pollution indicator.

INTRODUCTION

Amphibians possess moist skin, a combination of cutaneous and pulmonary respiration, and permeable tissue, making them highly sensitive to environmental conditions. Among them the Bullfrog (*Hoplobatrachus tigerinus*) is frequently used in histology, toxicology, physiology, and pigmentation research due to its hardy nature and easily identifiable organ system (Wells, 2007).

The bullfrog belongs to the family Dicroglossidae, they are the part of the order Anura, which encompasses frogs and toads, characterized by smooth skin, strong hind limbs, and a largely aquatic or semi-aquatic lifestyle.

The kidney of bullfrogs, being a mesonephric organ composed of glomeruli, tubules and collecting ducts is highly responsive to osmotic and toxic disturbance. They excrete urea which is an adaptive trait to conserve water in terrestrial environment. They show distinct morphological adaptations when exposed to harmful substances, reinforcing their sensitivity to waterborne changes. (Gehring et al., 2001)

Similarly, the lungs of amphibians consist of thin respiratory septa and vascular networks that are easily affected by the environmental fluctuations (Burggren, 1982). Melanophores, the melanin-containing pigment cells, are known to increase in number when the frog are exposed to environmental stresses (Elena S. Voronina, 2018).

Recent research studies in Brazil demonstrated that bullfrogs tadpoles accumulate metals in their tissues, reflecting the contamination levels of their environment. (De Almeida, 2024). These findings highlight the strong link between the water quality and amphibian physiology, emphasizing the importance of using frogs in environmental assessment. Amphibians serve as both a biological reference and a husbandry manual for understanding and sustaining amphibians under controlled conditions (F. Harvey Pough, 2007).

In the present study, we focused on the kidney and lungs of bullfrog organs, that play a crucial role in osmoregulation and respiration. The study aimed to compare the water quality parameters such as pH, dissolved oxygen, hardness, alkalinity, and temperature between normal pond water and the habitat from which the bullfrogs were collected, by comparing this we can understand how varying water characteristics may influence the physiological and structural integrity of amphibian tissue.

The biomonitoring approach not only provides insights into the adaptability of bullfrog but also serves as a monitor of the overall health of aquatic ecosystem they inhabit.

Aim

The aim of this study is to observe and analyze the histological structure of the kidney and lungs of the bullfrog (*Hoplobatrachus tigerinus*) with special reference to the presence of melanophores and their possible role as indicators of environmental stress.

Objectives

1. To collect and prepare kidney and lung tissues of the bullfrog for histological examination.
2. To study the microscopic structure of the kidney and lungs through processed tissue sections.
3. To identify and describe the occurrence of melanophores within these organs.
4. To compare the histological organization of the kidney and lungs and highlight their functional differences.
5. To evaluate the possible significance of melanophores as biomarkers of stress due to environmental or pollution-related factors.

MATERIALS AND METHODS

Experimental amphibian :

Hoplobatrachus tigerinus, bull frog are the largest frog. They have smooth, greenish-brown skin. They possess strong hind limb for jumping.

Frog collection

Bullfrog was collected from the pond in Nagarcoil on 15th September, 2025. A single adult male bullfrog was collected of 20cm length and 500 grams.

Tissue dissection

Bullfrog was euthanized ethically. Two tissues each from kidney and lungs were collected.

Tissue processing

Tissues were fixed in 10% neutral buffered formalin, dehydrated in graded alcohol, cleared in xylene, embedded in paraffin. Sections of 5-7 μ m thickness were cut and stained using Hematoxylin and Eosin.

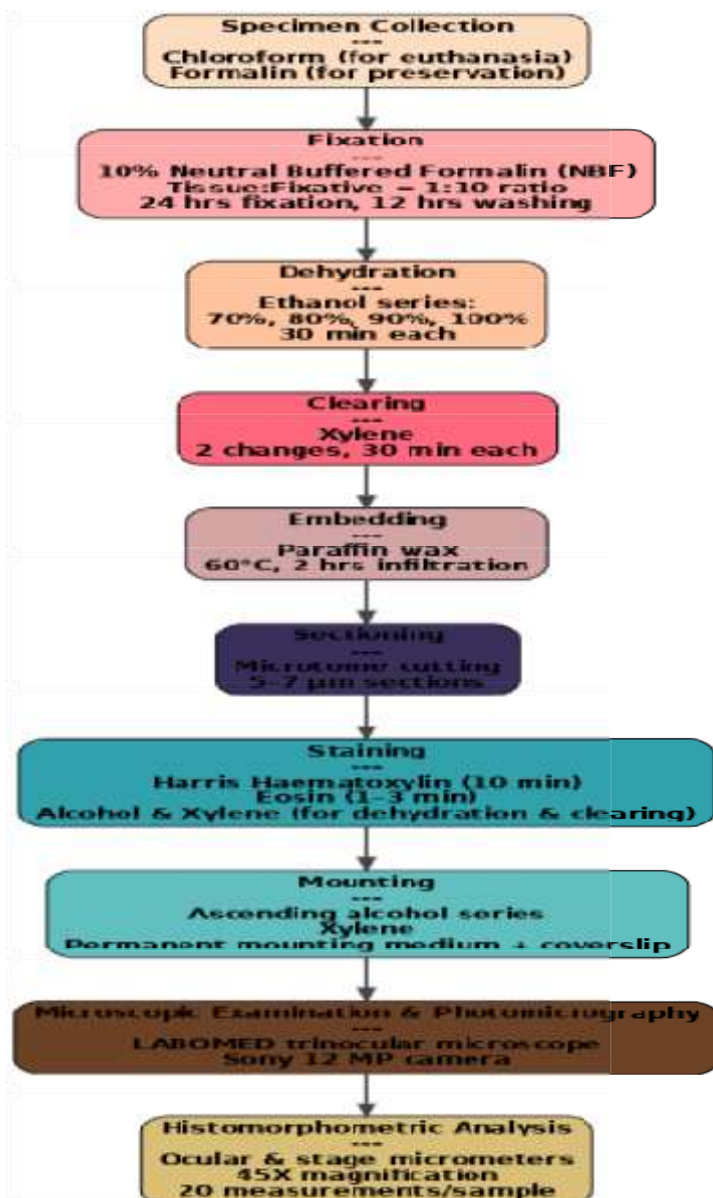
Microscopic analysis

The four was examined under the light microscope to observe

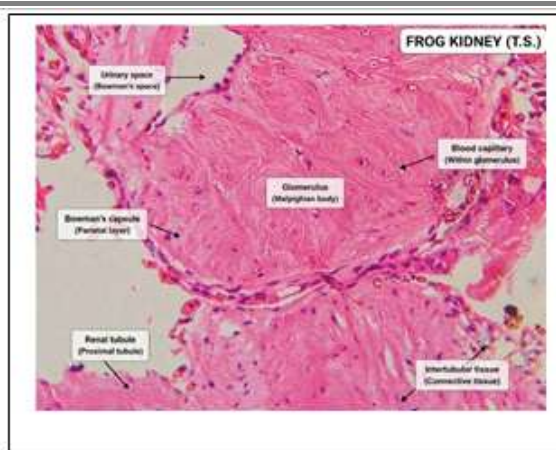
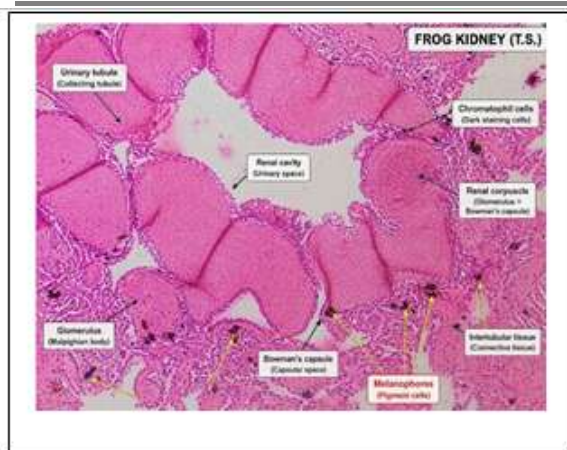
Kidney: lumen, glomeruli, renal tubule, melanophores, connective tissues

Lung; Alveoli, bronchiole, melanophore, connective tissue

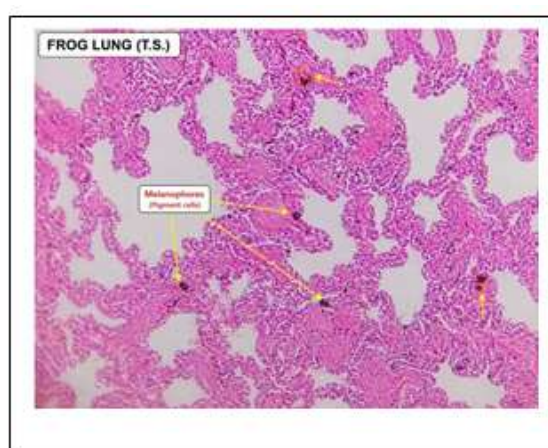
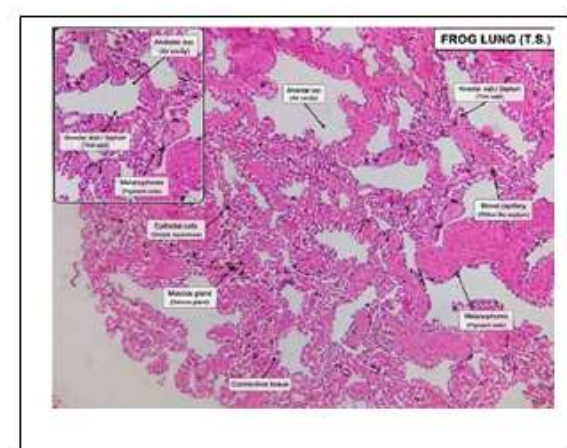
Histological sections of the kidney and lungs were examined under a light microscope at 45 $\times$ magnification. The major histological features of the kidney, including glomeruli, Bowman's capsule, renal tubules, lumen and melanophores, and the lung, including alveolar spaces, respiratory septa, vascular structures, connective tissue and melanophores, were examined. Histomorphometric measurements were obtained using an ocular stage micrometer, with 20 measurements recorded per tissue sample. The presence and distribution of melanophores in the kidney and lung tissues were qualitatively observed under a light microscope



Kidney Of Frog



Lungs Of Frog



Water sample collection

Normal pond water sample was collected and the habitat water sample in which the frog lived was also collected. Parameters such as pH, dissolved oxygen, hardness, alkalinity, temperature were tested using water quality analyzer.

RESULT

Water analysis confirmed that the frog habitat water differed from normal water, showing increased hardness, low pH, dissolved oxygen, alkalinity, and temperature than the normal pond water. These variations resulted in distinct histological differences.

Kidney of frog maintained in normal pond water has intact glomeruli, regular bowman's capsules compact cuboidal epithelium and normal lumen size. In frogs, exposed to the habitat water, kidney showed tubular dilation, cytoplasmic vacuolization, epithelial swellings, disrupted tubules, glomerular shrinkage and scattered necrotic area.

The lungs showed thickened alveolar septa, swollen, disorganized, vascular congestions, hemorrhagic capillaries, epithelial degenerations, suggesting compromised respiratory efficiency. Both the organs lungs and kidney contains numerous melanophores, the increase in pigment activity reflects that the frog was exposed to high stressful area like polluted area, pesticides or to any heavy metals. Melanophores were observed in both the kidney and lung tissues. Their presence and distribution were recorded as qualitative histological observations.

Water Quality Parameters

Parameters	Normal	Bullfrog	Optimal	
pH	Pond	Habitat	Range for Observation Amphibians	Remark
Dissolved	Water	Water	Both values fall within	Within optimal the range slightly lower than normal pond.
Oxygen (mg/L)	7.2	7.12 2.4	6.5 – 8.0 acceptable neutral range.	
Hardness	7.5	161	Habitat water DO	Significantly lower Oxygen, may stress aquatic life.
(mg/L)	90	7.3	> 5.0 is extremely low.	
Alkalinity (mg/L)	85	28	Habitat	Higher hardness in water habitat water, ideal above optimal range.
Temperature	26		50 – 150 exceeds hardness.	
(°C)			Habitat	water Very low is alkalinity in habitat water.
			20 – 120 alkalinity drastically low.	
			Both temperatures 20 – 30 within acceptable range.	Within normal range slightly warmer.

Water samples of 1 L were collected separately from the reference pond and the bullfrog habitat on 20 September 2025. The collected water samples were analysed using a water quality analyser. The parameters analysed included pH, dissolved oxygen, hardness, alkalinity and temperature. Each parameter was measured once, and the obtained values were recorded in their respective units.

DISCUSSION

The tubular swelling, glomerular shrinkage and necrotic patches seen in the kidneys resembles the degenerative changes documented by (Weyenberg, 1985), who noted that the amphibians renal tissues shows rapid deteriorations under chemical stress. (Czopek, 1962) also emphasized that amphibians kidney undergo functional impairment when environmental changes.

Lungs alterations such as thickened septa, epithelial swelling and hemorrhagic capillaries corresponds with the structural vulnerability described by (Gladys Hermida, 1998), (D.G.Smith, 1964) previously noted that the pulmonary vascular bed is highly sensitive to pollutants, and (Olitani & Naito, 1980) demonstrated that amphibians respiratory surface undergoes marked structural changes during environmental stress. These earlier observations support the pronounced respiratory damage recorded in the current study.

The melanophores activity inside kidney and lungs tissue shows the findings of (Elena Voronina, 2018), who documented the presence of internal melanophores during stress and the melanin provides protective antioxidant functions. Frogs when exposed to heavy metals also causes increase in melanophores (Kaczor - Kamiriska et al., 2020).

The observed presence of melanophores in the kidney and lung tissues may be associated with environmental stress; however, their density was not quantitatively measured in the present study. The present study was primarily based on qualitative histological observations, and statistical analysis was not performed. Quantitative histomorphometric analysis with replicated measurements and a larger sample size is recommended for future studies. However, the specific chemical contaminants responsible for the observed histological alterations were not determined in the present study.

CONCLUSION

The histological study of lungs shows thickened alveolar septa, swollen, disorganized, vascular congestions, hemorrhagic capillaries, epithelial degenerations and the kidney shows tubular degenerations, glomerular damage also the both organs contains numerous melanophores as a response to stress reflecting the organism attempt to cope with toxic exposure.

Overall the study of structural and functional alterations observed in amphibians organs, particularly kidney and lungs, serve a reliable indicators of ecological stress, The accumulation of melanophores, tissue thickening and cellular degenerations are the direct consequences of adverse environmental conditions. The comparative analysis of water quality parameters between the bullfrog lived pond water and the normal pond water revealed significant variations. The bullfrog habitat showed a lower dissolved oxygen content (2.4mg/l) and the higher total hardness (161mg/l) along with the difference in pH (7.12), alkalinity (7.3mg/l) and temperature of (28 degree celcius). These unfavorable parameters can directly influence the physiological functions of the frogs, including respiration and osmoregulation, leading to the histological changes in the lungs and kidney, hence the marked difference strongly supports that the bullfrogs are exposed to environmental stress within their habitat. The observed histological alterations and melanophore presence may be associated with environmental stress. However, the specific chemical contaminants responsible for these alterations were not determined in the present study

REFERENCE

1. Burggren, W. W. (1982). Pulmonary gas exchange in amphibians: Structural and functional perspectives. *American Zoologist*, 22(1), 1–16.
2. Czopek, J. (1962). Vascularization of the respiratory surfaces in Caudata. *Journal of Morphology*, 110(2), 215–230.
3. De Almeida (2024). Metal accumulation in bullfrog tadpoles as an indicator of aquatic contamination. *Brazilian Journal of Herpetology*, 28(1), 21–30.
4. Elena S. Pshennikova., & Anna S. Voronina. (2018). Melanophores inside frogs: Structure and functional significance of pigment cells in amphibian skin. *Journal of Evolutionary Biochemistry and Physiology*, 54(6), 450–458.
5. Gehring, W., & Masopust, J. (2001). The kidney in amphibians: Morphological adaptations and functional implications. *Journal of Morphology*, 250(3), 145–160.
6. F. Harvey Pough. (2007). Amphibian biology and husbandry. *ILAR Journal*, 48(3), 203–213.
7. Hermidia, G. N. (1998). Structural analysis of lungs in the common toad. *Herpetological Review*, 29(4), 157–166.
8. Kaczor-Kamińska, M., & Piotr, S. (2020). Multidirectional changes in sulfur metabolism in frog tissues exposed to heavy metals. *Ecotoxicology and Environmental Safety*, 195, 110–122.
9. Ohtani, O., & Naito, M. (1980). Renal microcirculation of the bullfrog (*Rana catesbeiana*): A scanning electron microscopic study. *Microvascular Research*, 20(1), 55–68.
10. Rant., & M. M. Stewart. (1943). The kidney and excretion in amphibians. *Biological Reviews*, 18(1), 1–32.
11. Smith, D. G. (1964). Pulmonary vascular bed structure in amphibians. *Journal of Anatomy*, 98(1), 33–46.
12. Voronina, E. S. (2018). Melanophore distribution and stress-induced pigmentary changes in amphibians. *Pigment Cell & Melanoma Research*, 31(4), 512–520.
13. Wells, K. D. (2007). *The Ecology and Behavior of Amphibians*. University of Chicago Press, pp. 1–1148.
14. Weyenberg, G. (1985). Ultrastructural characteristics of amphibian renal tissue under pollutant exposure. *Acta Zoologica*, 66(4), 243–260.