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Histological Analysis of the Gill and Swim Bladder of Hong Kong Catfish (*Clarias fuscus*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research aims to characterize the histological features of the gills and swim bladder of adult Hong Kong catfish (*Clarias fuscus*) weighing an average of 650 g and 40 cm in length. The organs (gills and swim bladders) were dissected and fixed for 48 hours in 10% neutral buffered formalin (NBF) fixative. The organs were then subjected to standard procedures: paraffin embedding, microtome sectioning, staining and mounting, followed by observation under a Carl Zeiss binocular compound microscope. The Gills of *Clarias fuscus* showed normal primary and secondary gill lamellae. A significant number of lymphocytes infiltrated the gill lamellae. The swim bladder tissues of *Clarias fuscus* displayed no inflammation, edema or necrosis in the lateral body wall and coelomic cavity. The columnar epithelium has expanded, folded, and filled the swim bladder's lumen

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entirely. The data acquired can be used to establish a baseline for healthy Hong Kong catfish, aiding researchers in understanding how gill and swim bladder respond to diseases and environmental factors.

Keywords: Histology; gill; swim bladder; Clarias fuscus.

1. INTRODUCTION

Histology offers comprehensive details on the microscopic structure of fish organs and tissues. Fish health can be evaluated and diseases can be diagnosed with the use of histological examination. Findings from a histological evaluation can shed further light on the physiological and/or environmental challenges that fishes experience in their natural habitat (Short and Meyers, 2001). Yonkos et al., (2000) emphasizes that histological changes in target organs, which are not always caused by toxicants, require a basic understanding of typical tissue structure to identify lesions. Therefore, knowledge and comprehension of an organ's typical histological structure are necessary for an appropriate histological evaluation.

Catfishes are widely consumed freshwater fish, making them highly popular globally (National Fisheries Institute, 2017). The family Clariidae, belonging to the class Actinopterygii and order Siluriformes, is widely distributed in Asia and Africa (Tian et al., 2023). The Hong Kong catfish, *Clarias fuscus* is mostly found in subtropical regions of China, Vietnam, Philippines and Hawaii (Bailly et al., 2009) and also imported into other parts of the world. The fish is valued for its high adaptability, rapid growth, and tasty flesh, and a popular choice among farmers and consumers. *Clarias fuscus* has been inadequately characterised since Lacepède, (1803) named *Macropteronotus fuscus* after a Chinese figure. The fish has a smooth, scaleless body with a sticky texture. The stomach is yellowish-brown or dark grey, and lateral white spots are present along the sides (Zheng and Pan, 1990). *Clarias fuscus* is sometimes mistaken with channel catfish, however it can be distinguished by its rounded tail and large dorsal and anal fins. As nocturnal feeders, they consume a variety of foods, including crustaceans, worms, insects, rice bran, leaves and organic waste and several other things (Yamamoto and Tagawa, 2000). Because of their robust physiology adaptation, diverse diet, ability to endure transportation over long distances and high economic value, they have the capability to be the ideal species for aquaculture (Kottelat, 2001; Nakabō, 2002).

Besides their nutritional and economic importance, the gills and swim bladder of *Clarias fuscus* play critical roles in physiological functions, making them key subjects for histological study. In addition to respiration, the fish gills also regulate osmoregulation, acid-base balance and the excretion of nitrogenous waste. The gills of fishes are very sensitive to any changes in water quality because the gill lamellae and the gill filaments offer a vast surface area for direct and constant interaction with water pollutants (El-Serafy et al., 2009; Au, 2004). Histological biomarkers found in fish gills can be used to assess the general health of the species and indicate the effects of exposure to various man-made contaminants (Wijeyaratne and Pathiratne, 2006).

The swim bladder in teleosts is an internal organ that is filled with gas and covered with a capillary network. It helps in buoyancy control, pressure reception, respiration (gas exchange) and the generation and reception of sound (Alexander, 1966; Yang et al., 2018). The primary cell types in the swim bladder include gas-gland cells, mesenchymal cells, smooth muscle cells and epithelial cells (Prem and Pelster, 2001; Winata et al., 2009). Histological investigations can potentially reveal pathological alterations in the swim bladder induced by stressful environmental factors. For instance, exposure to pollutants may result in thickening of the swim bladder wall, epithelial damage and even necrosis in some fishes. These changes have an impact on the health and buoyancy control of fishes.

Histology of Clariidae have been documented for *Clarias macrocephalus* (Tan-Fermin et al., 1997), *Clarias magur* (Muduli et al., 2021) and *Clarias batrachus* (Mishra and Behera, 2023). The histology of *Clarias fuscus*, a common catfish in Southeast Asia, have not been reported. So, this study aims to investigate the histology of gill and swim bladder of the Hong Kong catfish.

2. MATERIALS AND METHODS

2.1 Animal Collection

Healthy Hong Kong Catfishes (*Clarias fuscus*) with an average weight of 650 g, standard length of 36 cm and total length 40 cm were collected from Sathankuppam lake (11.700964º N, 79.691922º E), Cuddalore, Tamil Nadu - 607003. With a thick plastic bucket and an appropriate oxygen source, the fishes were transferred to the laboratory and then acclimatized in glass aquarium tank equipped with aeration tube with air stone. The fish were dissected to obtain the gills and swim bladders.

2.2 Histological Investigation

The histological method by Ma et al., (2016) was followed. The organs such as gills and swim bladders were immediately fixed in 10% neutral buffered formalin (NBF) fixative and stored in screw cap bottles containing the fixative. The initial fixation period was forty eight hours. Thereafter the samples were undergone for tissue processing. The tissues were dehydrated by immersing them in successive concentrations of alcohol (70%, 80%, and 90%) for one hour each, followed by two hours in 100% alcohol, and then cleared using Xylene-01 and Xylene-02 for one hour each. Then the tissues were impregnated for one hour each in two changes of paraffin wax prior to embedding. A manual rotatory microtome was used to cut the transverse sections to a thickness of 5 µm. Following five minutes of deparaffinization in Xylene-01 and Xylene-02, the sections were hydrated through a progressive series of alcohol from higher grade to lower grade – 100% EtOH (Ethanol), 90% EtOH, 80% EtOH, 60% EtOH each for 15 dips, followed by two minutes in distilled water. The tissues were stained using Hematoxylin and Eosin stain. Applying the routine procedure, stained sections were dehydrated by dipping it in 90% EtOH for 10 dips and then again 10 dips each in two changes of 100% EtOH and cleared by dipping it 10 times (dips) each in two changes of Xylene. Finally mounted with glass cover slip using Resinous mounting media (D.P.X). The histological sections were examined using a Carl Zeiss binocular compound microscope.

Fig. 1. Dissected Gill of *Clarias fuscus*

Fig. 2. Dissected Swim bladder of *Clarias fuscus*

3. RESULTS

3.1 Histological Study

3.1.1 Gill

The overall histological structure of the gills of *Clarias fuscus* showed normal organization, with lamellar epithelium. Normal primary and secondary gill lamellae were seen. Gills showed slight proliferation of the covering epithelium, notably at the base of the secondary lamellae, as well as a little infiltration of lymphocytes. The majority of the gills exhibited mild sloughing of epithelial cells from the secondary lamellae. A moderate quantity of lymphocytes infiltrated the gill lamellae (Fig. 3).

3.1.2 Swim bladder

The histological structure of the swim bladder tissues in *Clarias fuscus* showed no signs of necrosis, edema, or inflammation in the lateral body wall or coelomic cavity. The swim bladder was filled with gas. Desquamated epithelial cells in the distinct lumen of the vesicle. A primordial swim bladder with the gas gland, composed of low-columnar epithelial cells also visible. Connective tissue connects the lateral body wall and coelomic cavity. Gas gland cells appeared in the inner wall of the swim bladder. The columnar epithelium has increased, folded and completely filled the lumen of the swim bladder (Fig. 4).

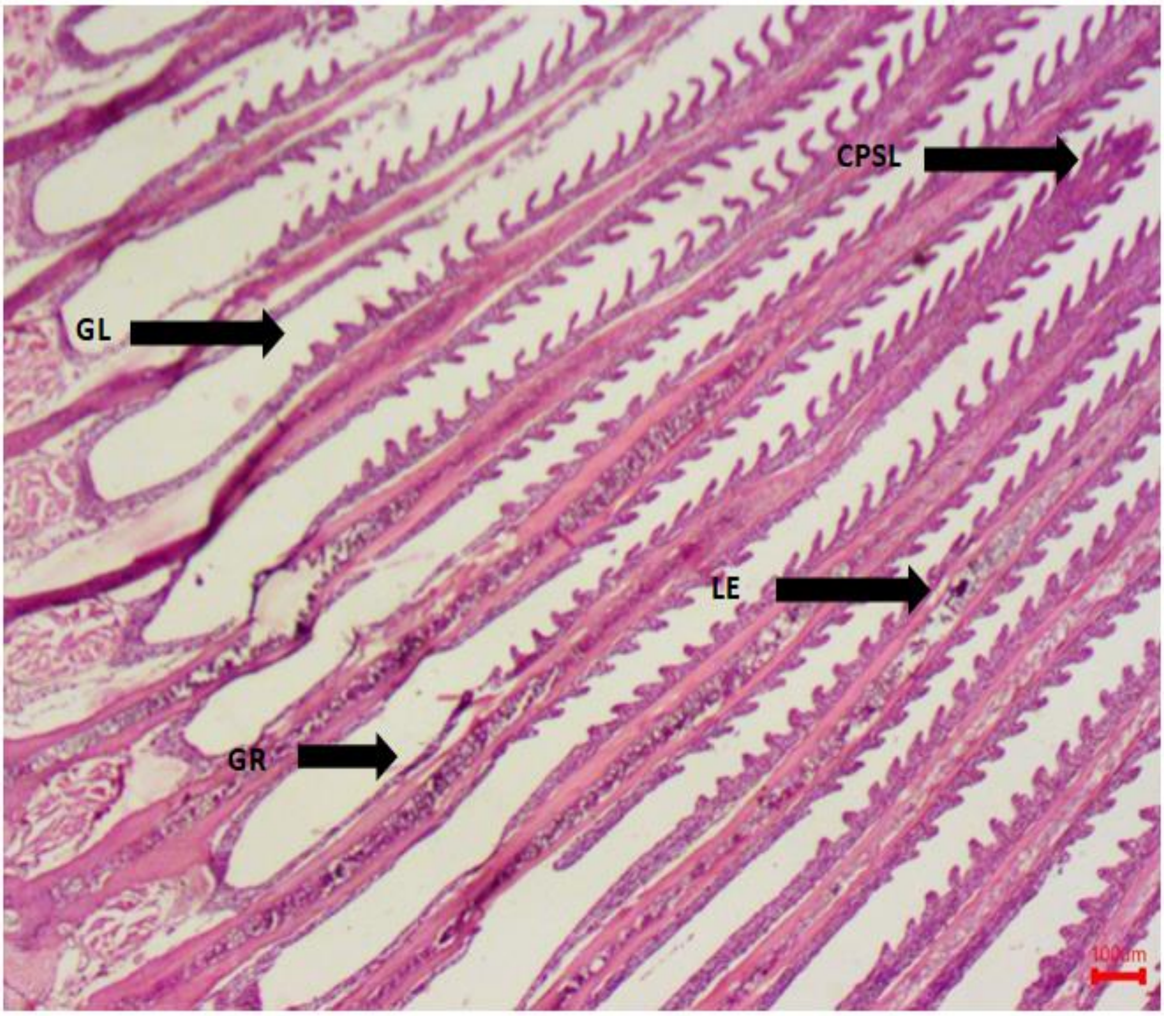

Fig. 3. Photomicrograph showing the Histology of Hong Kong Catfish (*Clarias fuscus*) - Gill stained with Haematoxylin and Eosin (Magnification 40X)

GL - Gill lamellae, GR - Gill Rachis, LE - Lamellar Epithelium, CPSL - Clogging of Primary and Secondary lamellae

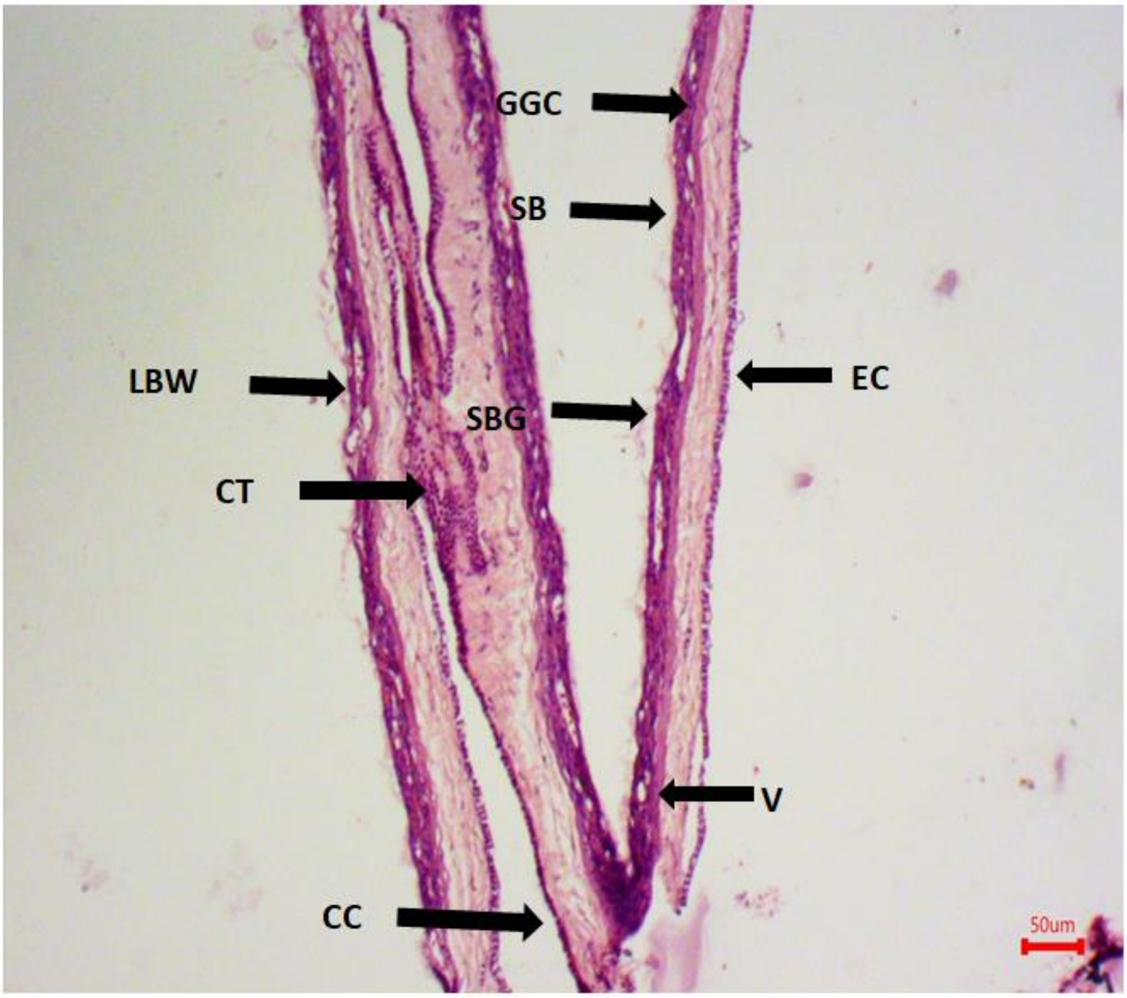

Fig. 4. Photomicrograph showing the Histology of Hong Kong Catfish (*Clarias fuscus*) – Swim Bladder stained with Haematoxylin and Eosin (Magnification 40X)
SB - Swim Bladder, SBG - Swim Bladder filled with Gas, GGC - Gas Gland Cells, LBW - Lateral Body Wall, CC - Coelomic Cavity, EC - Epithelial Cells, V – Vesicle, CT - Connective Tissue

4. DISCUSSION

The gills are considered a primary target of contaminants due to their role in vital processes such as respiration, osmoregulation, and excretion, as well as their continuous interaction with water, which increases susceptibility to changes in water quality (MN, 2003; Poleksić and Mitrović-Tutundžić, 1994). Catfishes, such as the Hong Kong catfish (*Clarias fuscus*), each gill comprised of a primary filament and secondary lamellae. An air-breathing catfish has hollow chambers inside both gills that hold the fish's air-breathing organs — a complex of branched, nearly tree-like structures packed with blood vessels. When the fish utilises its gills, these hollow chambers are frequently protected from water by a row of filaments, or strands of tissue (Harvey, 2014). The overall histological structure of *Clarias fuscus* gills revealed a lamellar epithelium with normal organisation. Similar structure was reported in the gills of *Clarias gariepinus* (Doherty et al., 2016). In our study, we observed normal primary and secondary gill lamellae in the gills of *Clarias fuscus*. A recent study was reported where *Clarias gariepinus* was cultured in water with a salinity of 0-ppt displayed normal gill structure with typical primary and secondary gill lamellae (Dawood et al., 2022). Also a similar structure was observed in the control group of *Heteropneustes fossilis* (Khatun et al., 2016), *Ictalurus punctatus* (Shahid et al., 2020), *Pangasionodon hypophthalmus* (Hossain et al.,

2022) and *Clarias batrachus* (Mishra and Behera, 2023).

The swim bladder of teleosts is a large, well-vascularized organ that is believed to be evolutionary similar to the lungs (Alexander, 1966; Nguyen, 2015). The swim bladder serves as both hydrostatic organ and a gas container, helping the fish to stay upright in the water column. It also acts as a resonating chamber for producing or receiving sound. The term “physostome” refers to adult fish with a pneumatic duct connecting their swim bladder to their gut, whereas “physoclist” refers to fish with a closed swim bladder (Zaccone et al., 2012). Physostomous fishes, such as catfish, includes a pneumatic duct that physically links the swim bladder to the esophagus, allowing gas to pass via the alimentary canal. This structure allows opportunistic microorganisms to penetrate the physostomous duct, potentially causing infections associated with poor water quality (Newton, 2019). In our investigation, we observed that the gas gland in the swim bladder of *Clarias fuscus* is composed of low-columnar epithelial cells. Silimar cell structure was found in the swim bladder of *Anguilla Anguilla* (Zwerger et al., 2002) and *Carassius auratus* (Zaccone et al., 2012. We found that the connective tissue of the swim bladder of *Clarias fuscus* continues across the whole swim bladder mucosa and connects the lateral body wall and the coelomic cavity which is also found in the swim bladder of *Oncorhynchus mykiss* (Villasante et al., 2019). The histological examination of fish gill and swim bladder is essential for comprehending health, reactions to environmental changes and respiratory and buoyancy adaptations. This research helps to quantify the impact of pollution, diseases and physiology, hence helping conservation and aquaculture management efforts for sustainable fish populations. Future research could look at the effects of environmental contaminants on the histological structure of *Clarias fuscus* gills and swim bladder, compare histological changes throughout developmental stages, and study adaptive mechanisms in different environments.

5. CONCLUSION

This is the first histological investigation of the gills and swim bladder of *Clarias fuscus*. This study provides baseline histological features for *Clarias fuscus*, serving as a reference for future research on environmental stressors and diseases, and facilitating the identification of biomarkers for environmental monitoring. By describing the normal histological characteristics of the gill and swim bladder, this work provides a reference for diagnosing pathological alterations in these organs. This is essential for developing diagnostic tools, analysing disease development and assessing the efficacy of treatments in *Clarias fuscus* and similar species.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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