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### Special Issue:

Emerging and Re-emerging Animal Health Challenges in Low and Middle-Income Countries

# Histological Characterization of Normal Gill Tissue of Oscar Fish and Goldfish

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**Abstract** | The aquatic biology is considered one of most biologically diverse, in the nature due to its ability of adaptation with different environmental conditions. These adaptations are compatible with the aquatic body needs to use minimum energy. According to the Oxygen requirements, some of the fish appeared active and other appeared inactive. The gills are the major site of external environment adaptation. Although the gills are very important in fish respiration, the literature on the histological aspects of gills is limited to a few types of fish. In this study, two types of fish (Oscar fish and goldfish) were used; the Oscar fish and goldfish. The investigated morphological and histological studies were done on the characteristics of gills on both types of fish. The results showed that the gills of the Oscar fish had a big surface area compared with the surface area of gill in goldfish. The gills of Oscar fish had a modification to get a high amount of oxygen using less energy. The study concluded that the active fish need a big surface area on their gills to get high oxygen and it adapted to survive the external conditions.

Keywords | Oscar fish, Goldfish, Gill histology, Astronotus ocellatus, Carassius auratus

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# **INTRODUCTION**

Fish are considered one of the largest and most important groups of vertebrates that inhabit the aquatic environment and are located at the top of the consumers in the food chain in the aquatic ecosystem (Dallinger et al, 1987). Gills play a major role in fish respiration, depending on the structures in which they are found, especially the secondary gill plates, which are rich in blood vessels and respiratory cells. The gills are the effective sites in the process of exchanging respiratory gases between the external environment (water) and the internal environment (blood) through these plates (Geherk, 1987; Swain and Richardson, 2010; Pathan et al., 2010). The structure and appearance of gills depend on the manner of the life of fish under the aquatic media, and it relates to the metabolic requirements of the fish (OIson, 2002). The fish are different in their motion activity. The fish with high motion activity have a high metabolic rate and tend to have a big respiratory surface area compared with fish with low motion (Suzuki et al., 2008; Hughes and Al-Kadhomiy, 1986; Evans et al., 2005).

The locomotion activity of the fish is considered one of many daily activities that depend on several aspects such as the morphological measurements of the movement (including the depth, aspect ratio of the caudal fin, acceleration, and the caudal peduncle), the alimentary trunk contents during the locomotion, and the area of the

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respiratory gills (Gutierrez and Martorella, 1999; Wells et al., 2005).

The respiratory area effective of the fish's gills relies on gas attachment ( $O_2$  and  $CO_2$ ) and the amount of water pumped into the gills system (Duthie and Hughes,1987). The amount of oxygen in the fish reflects the activity rate of the fish. For example, the Scomber fish (an active fish) has an oxygen capacity of around 19.6% while the *Opsanus tau* (which considered inactive fish) has an Oxygen capacity of 5.3% (Mcfarland et al., 1979).

By measuring the respiratory area of the gill, scientists can calculate the amount of oxygen consumption for respiration, metabolism, and growth. Researchers has found that the amount of oxygen consumption was higher in fish like *Onchorhynchus* which was 800 mg while the *Carassius auratus* had 170mg/h. This indicates that the fish with high speed and high locomotion activity rate need a large amount of oxygen to fulfill their requirements compared with the fish with low activity rate which did not need to move for a large area since they had everything from food and other nutrients in the environment nearby (Marsden, 1991; Alexander, 1974; Smith et al., 2012; 2007).

Astronotus ocellatus had been described for first time in 1831 by Dr. Jean Louis Rodolphe Agassiz. First, he had described it under the name *Lobotes ocellatus* because he thought it was a salty water fish but then he classified its species under the genus *Astronotus* (Table 1) (Firouzbakhsh et al., 2011).

**Table 1:** Classification of the Oscar fish *Astronotus ocellatus*according to (Agassiz, 1831).

Kingdom	Animalia
Phylum	Chordata
Class	Actinopterygii
Order	Perciformes
Family	Cichlidae
Sub family	Astronotinae
Genus	Astronotus
Species	A. ocellatus

The Oscar fish is carnivorous. The mainly feed on small fish, aquatic insects, and Crustacean are the main food of Oscar fish (Soares et al., 1986; Honebrink, 1990, Invertebrate).and fish also feed on everything falls in the water can be source of food to oscar fish (Consoli, 1991; Kullander, 1986). The goldfish *Carassius auratus* is beyond to the Carp family (Table 2) which lives in the fresh water (Linnaeus, 1758). The origin place of the goldfish is China after that from China to the world. The goldfish is one of most fish types which breeds and raises in houses due to

its ability to live in freshwater and light saline water like rivers, ponds, and lakes. The goldfish's food is suspicious material and invertebrates located in the bottom of the water. The breeding season began in spring. The goldfish put their eggs on the aquatic plants and sometimes put the eggs more than one time (Joseph, 1980).

Table	2:	Classification	of	the	goldfish	according	to
(Linna	eus	, 1758).			0		

Kingdom	Animalia
Phylum	Chordata
Class	Actinopterygii
Order	Cypriniformes
Family	Cyprinidae
Genus	Carassius
Scientific name	Carassius auratus auratus

Although the gills are very important in fish respiration, the literature on the histological aspects of gills is limited to a few types of fish (Arey, 1974; Ojha et al., 1987). The gills are the site of gas and ion exchanges. Therefore, the gases and ions exchange depend on the gill surface area (4199 Pauly). Furthermore, the speed of blood and water flow through the plates of gills plays an important role in the gas exchanges. Since the amount of oxygen and the fish activity link with the surface area of the gills, the measurement of gill surface area became very crucial in determining the fish activity. Histologically, the gill lamellae consist of primary lamellae (gill filaments) and secondary lamellae (Wilson and Laurent, 2002). Active and fast-swimming fish have a large number of secondary lamellae per one millimeter. Also, the shape of the lamellae is characterized by small and narrow size such as in Tuna fish. On the other hand, the fish with low activity have fewer gills filaments and wide secondary lamellae with few numbers of them. There is the third group of fish that have a moderate number and size of lamellae called the fish with moderate activity like shank (Roubal, 1987).

Given the importance of gills in fish respiration, the histological characteristics of gills are only defined for limited species of fish. In this study, two types of fish (Oscar fish and goldfish) were investigated for morphological and histological characteristics of gills with an aim to advance the literature on this important aspect of locomotion in Oscar fish and goldfish.

### MATERIALS AND METHODS

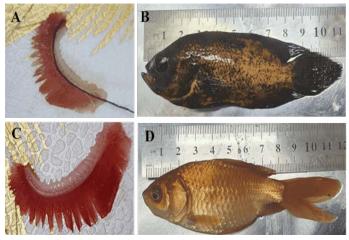
#### EXPERIMENTAL ANIMALS Morphology study

To calculate the surface area of the gills, first gills were taken out from the left side. Next, they were separated and

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#### Journal of Animal Health and Production

washed with tap water. Then, the gills were measured by putting them on anatomical plates (Figure 1A, B, C, D) following the (Hughes 2000).



**Figure 1:** A and B measuring the surface area of gills in Oscar fish. C and D measuring the surface area of gills in Goldfish.

The approach utilized for measuring gill surface area involved several steps. Firstly, the length of the gill arch was determined by rounding it to the nearest millimeter using a soft wire to replicate the arch's shape and measuring it. Next, the number of gill filaments was counted under a dissecting microscope. Subsequently, the mean length of gill filaments for each gill arch was calculated. This involved measuring every tenth gill filament if the total number of filaments was less than 100, or every twentieth filament if the count exceeded 100. The average number of gill filaments for each arch (totaling four arches) was then determined, followed by measuring the mean length of gill filaments for each arch.

To ascertain the number of secondary lamellae, the gill filaments from the second and third arches were gently scratched and immersed in a physiological solution of NaCl 9%. Subsequently, the samples were observed under a light microscope to count the number of secondary lamellae per millimeter of gill filament. This method follows the equation outlined by Hughes (2000). As following:

#### $A = L \times N \times B1$

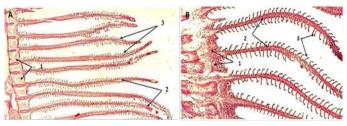
A= the surface area of the respiratory gill (mm<sup>2</sup>); L: the length of the gill filaments; N: the number of the gill lamellae per one millimeter of the gill filament; B1: the surface area of the single lamellae.

#### HISTOLOGICAL STUDY

Twelve healthy fish (6 Oscar fish and 6 Goldfish) were brought into laboratory alive for the histological study. The fish were monitored for two weeks before doing further experiments. After fish had been sacrificed, gills were collected. Next the samples were washed and fixed using 10% formalin for 24 hours. Then the samples were histologically processes to prepare samples for sectioning. The histological processes were according to Bancroft and Steven (1982). In summary, the samples were dehydrated using ethanol 35%, 50%, 70%, 80%, 90% and 100% for one hour for each concentration. Next, clearing the samples suing xylene. Then, the samples were infiltration and embedding using paraffin wax. After all the steps above, the samples were kept in room temperature for cooling. The samples then were sectioned and stained using routine stains (Hematoxylin and Eosin).

### **RESULTS AND DISCUSSION**

Fish activity is a primary indicator for fish health. To determined fish activity, measuring the gill surface area is one of most effective methods. The surface area of gill is the site of ions and gases exchange (Pauly, 1989). This agrees with our results that showed the morphological study indicates a difference in the surface area of the gills. A. ocellatus fish have a larger gill surface area compared to C. auratus. This indicates that A. ocellatus fish need more gaseous exchange. Histological sections of the gills of both C. auratus (A) and A. ocellatus (B) show the following structures: The gill arch is the cartilaginous structure that supports the gill filaments. The gill filaments are the thin, feathery structures that absorb oxygen from the water. The lamina is the thin, plate-like structure that forms the gill filaments. The gill arch is clearly visible in both species and provides support for the gill filaments. The gill filaments are long and thick, with a large surface area for gas exchange in A. ocellatus compared to C. auratus. The lamina of A. ocellatus is tightly packed and has a smooth surface, indicating a high capacity for oxygen absorption. There are significant differences in the histological structure of the gills between the two species. From the above, the histological sections of the gills of C. auratus and A. ocellatus showed different structures, suggesting that both species have adapted to extract oxygen from water using similar mechanisms but with different efficiencies. The gill arch, gill filaments and lamellae work together to provide an efficient gas exchange system (Figure 2).



**Figure 2:** Histological section of Gills in (A) *Carassius auratus* and (B) *Astronotus ocellatus* show (1) gill arch, (2) gill filaments, and (3) Lamella (H & E, 10X).

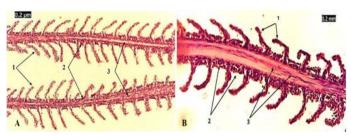
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**Table 3:** Morphological measurements of gills in goldfish and Oscar; (NG) number of gill filmants, (LG) Length of gill arch, (NA) Length of gill arch, (LR) length of gill filament, (AL) Area of lamella, (NL) number of lamella, and (TA) the totla surface area.

Types	AL	NL	LR	NA	LG	NG	Weight	Length	TA
Goldfish	0.0960	70	33	4	30	75	16	13	2777617.92
Oscar	0.1148	71	52	4	35	95	14	11.13	3406326

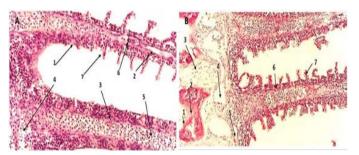
The histological study also confirmed that the difference in the tissue composition of the gills of both species. It was noted that the gill filaments were longer in *A. ocellatus* compared to the gill filaments of *C. auratus* (Table 3). This difference in the length of the gill filaments increases the surface area available for breathing (Figure 3). These finds were similar to the results of other studies were found that the length of gill filaments reflected the size of the gill surface area which in-turn the amount of the gases and ions exchanging. Also, it works in the osmosis pressure between fish body and external environment (Chen et al., 2022).



**Figure 3:** Histological section of gill filaments in (A) *Carassius auratus* and (B) *Astronostus ocellatus* show (1) secondary lamellae, (2) filament epithelium, and (3) venous sinus (H & E, 20X).

Histological sections of the gill arch of both C. auratus (A) and A. ocellatus (B) show structures that are similar in appearance with differences in fine detail. The gill arch is a cartilaginous structure that supports the gill filaments. It is prominent in both species, averaging 450  $\mu$ m in C. auratus and 500 µm in A. ocellatus. The gill comb is more prominent in C. auratus, indicating a stronger support for the gill filaments. The submucosa is a layer of connective tissue that supports the epithelial lining of the gill arch. It is thicker in both species, averaging 200 µm in C. auratus and 250 µm in A. ocellatus. The submucosa is thicker in A. ocellatus, indicating a richer blood supply to the gill arch. Adipose tissue is present in the gill arch, indicating energy storage. It is more abundant in A. ocellatus, with an average area of 300  $\mu$ m<sup>2</sup>, than in *C. auratus*, with an average area of 200 µm<sup>2</sup>. Adipose tissue is more abundant in A. ocellatus, indicating greater energy storage capacity (Figure 6). The efferent gill arteries are blood vessels that carry oxygenated blood away from the gills. They are clearly visible in both species, with an average diameter of 50 µm in C. auratus and 60 µm in A. ocellatus. The afferent gill artery is a blood vessel that carries deoxygenated blood to

the gills. It is also clearly visible in both species, with an average diameter of 100 µm in C. auratus and 120 µm in A. ocellatus. The efferent and afferent gill arteries are larger in A. ocellatus, indicating greater blood flow capacity. The primary lamellae are the main structural units of the gill filaments. They are tightly packed in both species, with an average length of 900 µm in C. auratus and 1000 µm in A. ocellatus. The secondary lamellae are smaller, branched structures that increase the surface area for gas exchange. They are also tightly packed in both species, with an average length of 450 µm in C. auratus and 500 µm in A. ocellatus. The primary and secondary lamellae are tightly packed in A. ocellatus, indicating a high capacity for gas exchange. The increase in cell size also increases the surface area for breathing, and this increases the process of gaseous exchange in A. ocellatus. The gill filaments had primary and secondary lamellae (Figures 4 and 5) as reported earlier by Evans et al. (2005), Randall (1982) who have noticed that Bony fish gills consisted of four gills and each one divided into two halves (Hemibranchs) which separated



**Figure 4:** Histological section of Gill arch in (A) *Carassius auratus* and (B) *Astronotus ocellatus* show (1) gill raker (2) submucosa, (3) adipose tissue, (4) efferent branchial arterioles, (5) afferent branchial artery, (6) primary lamellae, and (7) secondary lamellae (H & E, 40X).

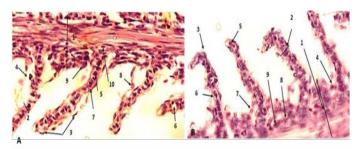
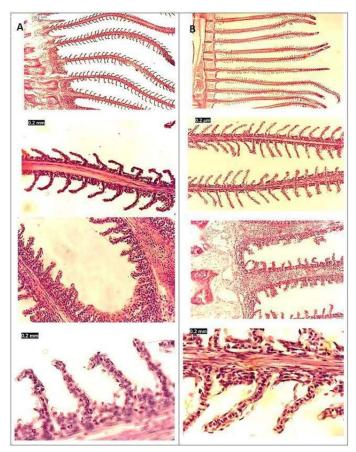


Figure 5: Histological section of Gill arch in (A)*Carassius auratus* and (B) *Astronotus ocellatus* show (1) Primary lamella, (2) secondary lamella, (3) epithelial cell, (4) mucous cell, (5) pillar cell, (6) lacuna (*capillary lumen*), (7)

erythrocyte within capillary lumen, (8) chloride cell rodlet cell, (9), and (10) undifferentiated basal cell (H & E, 100X).



**Figure 6:** The histological differences between the *Astronotus ocellatus* (A) and *Carassius auratus* (B) (H & E).

posteriorly and attached anteriorly. Furthermore, the arch gill, which play role in the management of pressure inside the gill and worked in the organizing the water movement inside gill, contained the gill rakers that prevented the food micro elements from passing the gill and work as a filtration (Wilson and Laurent, 2002). They have investigated that the filaments had two types of lamellae including the primary lamella and the secondary lamella that consisted of one layer of epithelial layer to work as a site of gas exchange.

### CONCLUSIONS AND RECOMMENDATIONS

Histological sections of the gills of Carassius auratus and Astronotus ocellatus show rather similar phenotypically structures, suggesting that both species have adapted to extract oxygen from water using similar mechanisms. However, there are some differences in the size and abundance of certain structures, suggesting species-specific adaptations related to their ecology, behavior and lifestyle.

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### **NOVELTY STATEMENT**

This research unveils a groundbreaking discovery in the structural complexity of fish gills, highlighting a previously unrecognized microstructural adaptation that enhances oxygen extraction efficiency. Our study identifies a unique pattern of lamellar arrangement and ciliary movement within the gill filaments that significantly improves respiratory function under varying environmental conditions.

# **AUTHOR'S CONTRIBUTION**

HMA: conceptualized the study, designed the research methodology, and supervised the experimental work. He also led the data analysis and interpretation and was the primary writer of the manuscript. SKA: Contributed to the experimental design and conducted the majority of the laboratory work. She assisted with data collection, analysis, and provided substantial input into the manuscript writing and revision. FAA: provided critical insights into the microstructural analysis of fish gills and developed the specialized imaging techniques used in the study. Assisted with the literature review and provided expert advice on the theoretical implications of the findings. He helped draft sections of the manuscript and contributed to the final revisions.

#### **CONFLICT OF INTEREST**

The authors declare that there are no conflicts of interest related to this study on fish gill structure. None of the authors have financial or personal relationships with organizations or individuals that could influence the outcomes of this research. This research was conducted independently and funded through institutional grants without external sponsorship from companies or entities that could potentially benefit from the results. All authors have disclosed any potential conflicts of interest and have adhered to ethical guidelines to ensure the integrity and objectivity of the research.

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