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Original Article

Histological Structure of the Gills and Respiratory Surface Area of the *Trachurus Mediterraneus* Caught from the Coast of Misurata City

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Abstract *- The gills function to supply the body with the oxygen necessary for the physiological processes occurring in fish. Their efficiency varies depending on the species, and the gills' surface area is one factor that determines the fish's efficiency and activity level. A rotary microtome was used to prepare tissue sections of the gills of Trachurus mediterraneus, a species found along the coast of Misurata city, to study the histological structure and estimate the respiratory surface area during May 2024. The macroscopic examination revealed the shape of the gill arches, with an average of 131.75±0.5 gill filaments arranged in an orderly manner on the dorsal surface of the gill arch, with an average length of 38.75 ± 1.25 µm. The average number of primary lamellae was 229.5±57.44, with an average length of 2.21±0.43 µm. Histologically, the gill lamellae are covered by two layers of epithelial cells, while the secondary lamellae are covered by a single layer of epithelial cells, along with supporting cells within their structure. The gill filaments are supported by cartilage tissue, and there are several blood sinuses and lacunae. The absolute and relative respiratory surface areas of the gills of T. mediterraneus were 148.5888 m² and 0.425319, respectively. The histological structure indicates that the studied fish possess cartilage supporting the gill filaments and numerous blood sinuses. The primary lamellae are lined with stratified epithelial cells, while the secondary lamellae are lined with simple epithelial cells. Additionally, pillar cells are present, supporting the secondary lamellae and enclosing many blood spaces. The histological structure and micrometric measurements play a crucial role in understanding the physiological activity of fish, which is oxygen-dependent, and the structural and histological adaptations of the gill filaments.*

Keywords *- Fish, Gills, Filaments, Gill arches, Pillar cells.*

1. Introduction

The Mediterranean Sea is one of the seas rich in a wide variety of fish species, with an estimated 664 species, approximately 440 of which are found off the Libyan coast. The Mediterranean horse mackerel (*Trachurus mediterraneus*), also known as the horse mackerel, is one of the fish species found in the Mediterranean Sea. It is also present in the eastern Atlantic Ocean, from the Bay of Biscay to Mauritania (1). T. mediterraneus is a bony fish belonging to the family Carangidae. It is considered a pelagic migratory fish that forms schools and feeds on sardines, anchovies, and small crustaceans (1,2,3). Fish gills play a crucial role, particularly in physiological functions, by providing a significant surface area for gas exchange. They are also essential for eliminating nitrogenous wastes and maintaining acid-base ionic balance in the blood (4). Laurent et al. (5) and Mohammed (6) indicated that the structure and physiological function of the gills primarily depends on the surface area of the gills and the presence of numerous cells that play essential roles in ionic balance and regulation, such as chloride cells and goblet cells. In addition, fish gills are considered effective tools for biomonitoring due to their large surface area and their direct and continuous contact with the surrounding environmental medium (7, 8).

The gill arches bear a double row of primary lamellae on their dorsal surface, which support two layers of secondary lamellae located on the upper and lower surfaces (9, 10). The secondary lamellae play a role in increasing the surface area of the gills. The gill filaments extract gases through a thin epithelium composed of two layers situated on a basement membrane and surrounded by supportive interstitial cells known as pillar cell capillaries (11, 9).

Numerous studies have examined the histological structure and surface area of the gills in various fish species. The surface area of the gills has also been studied in various fish species. Samajdar and Mandal (10) examined the histological structure and surface area of *Labeo bata*, while Mohammed (6) conducted a study on Epinephelus coioides, and Samei et al. (12) also studied the histological structure of the gills in Nile tilapia. Additionally, Skeeles and Clark (13) investigated the surface area of the gills in *G. maculatus* larvae and its relationship with body mass and temperature. Phadmacanty et al. (14) studied four fish species: *Barbodes binotatus*, *Pterygoplichthys pardalis*, *Rasbora lateristriata*, and *Amphilopus citrinellus*. The study of the gill tissues and respiratory surface area of the Mediterranean horse mackerel is the first investigation of this species, particularly along the marine coast of Libya. Therefore, the current study aimed to investigate the histological structure and estimate the surface respiratory area of the gills of *T. mediterraneus.*

2. Materials and Methods

Five fish samples for the current study were collected from the coastal area of Misurata in May 2024. The fish were transported directly to the Zoology Laboratory at the Faculty of Science, Misurata University, using a plastic cooler insulated with crushed ice. The total body length (cm) and weight (g) were recorded, and the target species for the study was identified using the Fishbase (1).

The four-gill arches were extracted from the left side, washed with tap water, and then transferred to a dissection dish containing physiological saline for examination of the main components using a dissecting microscope. The length of each gill arch was measured to the nearest millimetre using a metallic wire (Figure 1).

The gill surface area (GSA) was measured using a microscope equipped with a digital camera (Olympus BX4, Olympus DP22, Olympus IMS, Waltham, MA, USA), following the methodology described by Schneider et al. (1**5**). The number of gill filaments on each arch on the left side was counted. The lengths of the first and last filaments and every tenth filament in between were measured to calculate the Total Filament Length (TFL).

The second-gill arch from the left side was used for histological examination and external measurements. The gill arch was passed through a graded series of alcohols, embedded in paraffin, and sectioned to a thickness of 5 μm. The sections were stained with hematoxylin and eosin (H&E) at the Tissue Laboratory, Misurata Center, Libya. Digital images of the target components were captured using a light microscope (Olympus BX43 equipped with an Olympus DP22 camera, Olympus IMS, Waltham, MA, USA). The number of secondary lamellae for each gill filament was counted, and the surface area of ten secondary lamellae was measured by multiplying the height by the width. The average surface area of the secondary gill lamellae was then calculated.

According to the Hughes equation **(**1**6) (**1**7),** GSA (mm^2) = TFL × N × Bl, the absolute gill surface area (mm^2) and relative gill surface area (mm^2/g) were calculated. Here, TFL represents the total number of double gill filaments, N denotes the number of gill lamellae per 1 mm of filament, and Bl is the diameter of the secondary lamellae of the primary gill filaments.

2.1. Statistical Analysis

Data were processed using SPSS software (version 19) through one-way analysis of variance (ANOVA) to determine differences between means and to assess the significance of differences (P>0.05).

4. Results and Discussion

4.1. Morphological Characteristics

structure of the four gill arches. Each gill arch consists of two main parts: the epibranchial (upper branch) or short upper limb, located at the roof of the pharynx, and the ceratobranchial (lower branch), a long lower limb that encircles the pharynx (Figure 1). The morphological examination of the gill arch indicates the presence of numerous fine gill filaments, which are located on the dorsal or external surface of the gill arch and appear pinkish.

Several gill rakers are attached to the ventral or internal surface of the gill arch, with their free ends directed towards the pharyngeal cavity (Figure 1). The structure of the gill arch is described as a supporting framework for the gill filaments, facilitating the direction and filtration of water flow as it passes through the pharynx (18). Furthermore, it supports the cartilaginous framework of the gills (19, 2024).

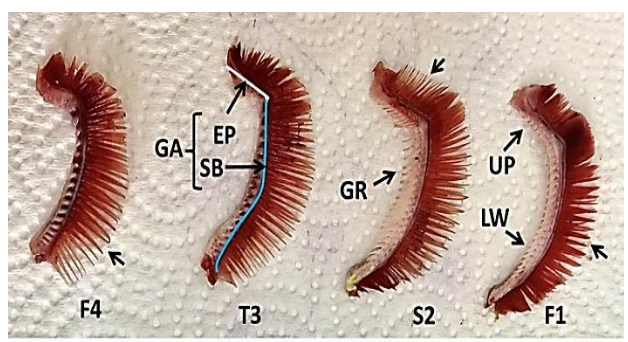
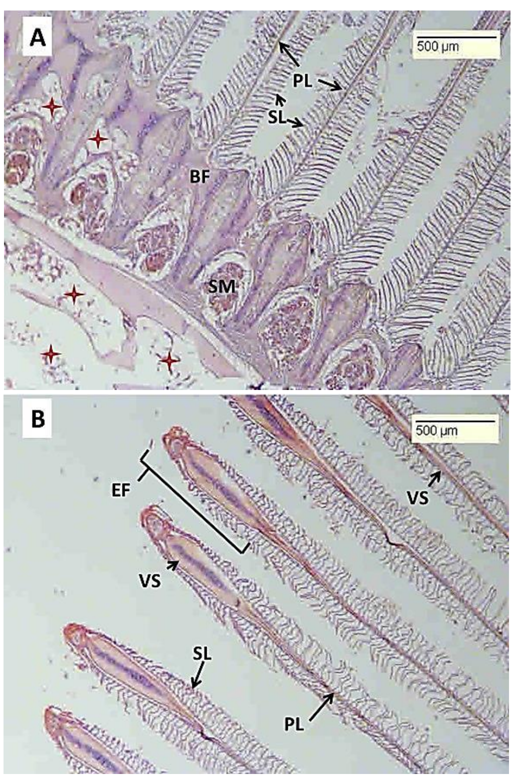


Fig. 1 Lateral view of the gill arches in *T. mediterraneus*. (UP) Upper limb, (LW) Lower limb, (Arrow) Gill filaments, (GR) Gill rakers, (EP) Epibranchial, (SB) Ceratobranchial, (GA) Gill arch, (F1-F4) Arrangement of the gill arches.

4.2. Histological Structure

The current study presents the histological structure of the gill filaments in *T. mediterraneus*, where primary gill filaments are observed to be arranged in parallel rows on the dorsal surface of the gill arch (Figure 1-A). Both surfaces of the primary gill filaments bear numerous secondary lamellae (respiratory secondary lamellae) arranged in a bilaterally symmetrical pattern resembling leaflets along each filament, extending from the base to the tip (Figure 2-A, B). The histological examination reveals the presence of blood sinuses located at the tips of the gill filaments. Additionally, a blood sinus is enclosed between the epithelial tissue and the supporting cartilaginous tissue of the gill filaments (Figure 2 – B, C, D). Blood cavities were also observed at the bases of the secondary gill filaments, along with numerous supporting cells (pillar cells) within the secondary lamellae, which enclose blood cavities (Figure 2 – D and Figure 3). These findings are consistent with numerous studies conducted on various fish species (1**3**, 20, 19). According to Cardoso et al. (21) and Wilson et al. (22), the epithelial cells of gill filaments are similar across fish species despite anatomical variations. Figures (4 and 5) illustrate the number of gill filaments in *T. mediterraneus*. The average number of gill filaments on the dorsal surface of each gill arch was 131.75±0.5. Additionally, the number of secondary lamellae per gill filament was 229.5±57.44, with this variation being statistically significant ($P>0.05$). According to the current study, the average length of the primary lamellae was 20.064±1.50 mm, while the average length of the secondary lamellae was 1.84 mm ($P<0.05$), with a thickness of 0.1 micrometres (Figure 4)



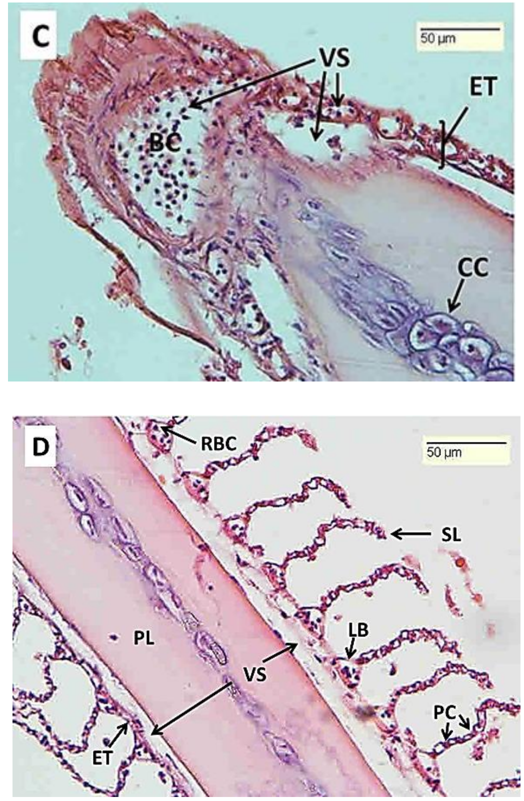


Fig. 2 A-D: Section through the gill filament of *T. mediterraneus*. (SL) Secondary lamella, (PL) Primary lamella, (BF) Base of the gill filament, (SM) Striated muscle, (VS) Blood sinus, (EF) Gill filament tip, (ET) Epithelial plate, (CC) Cartilage cells, (BC) Blood cells, (PC) Supporting cells, (LB) Blood cavity. (H&E).

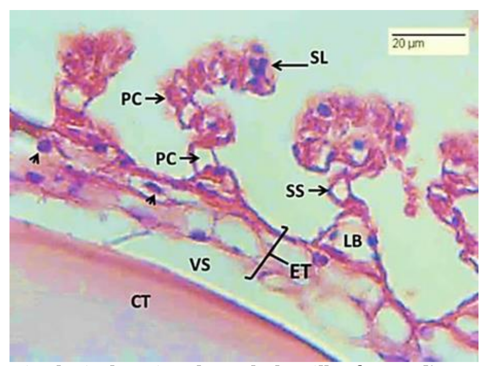


Fig. 3 Histological section through the gills of *T. mediterraneus*. (SL) Secondary lamella, (PC) Supporting cell, (LC) Cavity, (SS) Squamous cell, (LB) Blood cavity, (ET) Epithelial tissue, (VS) Blood sinus, (CT) Cartilaginous tissue. (H&E).

The current study indicates that the average length of gill filaments in *T. mediterraneus* was 20.064 mm, which is comparable to that of *Lophius piscatorius* (20.500 mm) but differs significantly from *Trachurus trachurus* (3.920 mm) (17). The morphometric measurements of bony fish gills, including gill filament length and the number and surface area of secondary lamellae, have a direct impact on the overall activity of the fish (Hughes, 1984; Roubal, 1987; Mansour, 1998; Skeeles and Clark, 2023). Fish are classified as active or inactive species based on their oxygen demand (19).

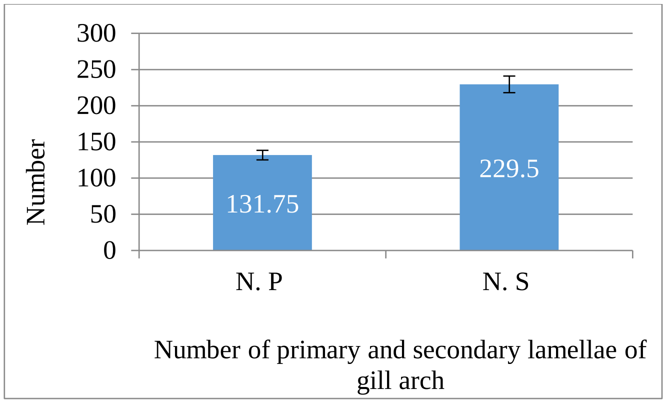


Fig. 4 Number of primary and secondary gill lamellae in *T. mediterraneus.*

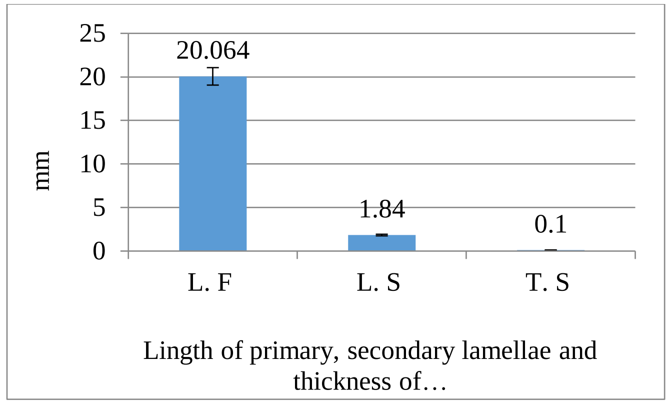


Fig. 5 Measurements in micrometres of primary and secondary gill lamellae in *T. mediterraneus*.

Using Hughes' equation, the total respiratory surface area of the gills in *T. mediterraneus* (1485.88 mm²) and the relative respiratory surface area (4.412 mm²/g) in the current study are comparable to the findings of Mohammed (2018) in his study of the gill surface area of *Epinephelus coioides*, which reported a total surface area of 7533.97±349.25 mm² and a relative surface area of 91.77±19.09 mm²/g. Similarly, in *L. xanthopterus*, the total and relative surface areas were found to be 6485.92±1.65 mm² and 140.47±1.50 mm²/g, respectively (23).

The high activity level of *T. mediterraneus* observed in the current study indicates a need for a large respiratory surface area to meet the high oxygen demand required for metabolic processes essential for this species. Suzuki et al. (24) noted that active fish with high metabolic rates have larger gill surface areas than less active fish with lower metabolic rates and smaller gill surface areas.

5. Conclusion

The results of the current study highlight the efficiency of the anatomical structure supporting the gill filaments, as well as the presence of respiratory epithelial cells and numerous cavities and blood sinuses. These features enable *T. mediterraneus* to obtain sufficient oxygen to perform vital physiological processes.

Additionally, the number of gill filaments in *T. mediterraneus* contributes to an increased respiratory surface area, placing them within the range of active fish species.

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