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Original article

Histological and Histochemical Characteristics of Some Organs of *Serioladumerili* Fishes from the Libyan Eastern Coast

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ABSTRACT

The paucity in the literature or published studies on the histological and information regarding the histological and histochemical features of Yellowtail (*Seriola dumerili*) on the Libyan eastern coast led to a study of the histological and histochemical structures of different organs (skin, gill, and liver) in *Seriola dumerili* fish from the Libyan eastern coast. Twenty male fish were collected twice (ten fish each) in March 2022 from two fisheries sites on the eastern Libyan coast. Immediately, skin, gill, and liver samples were collected and preserved in Bouin's solution, and then the paraffin sections were stained with H&E, Crossmon's trichrome, and PAS stain for histochemical and histological studies. Histological examination revealed that the skin of *Seriola dorsalis* consisted of epidermis, dermis, and hypodermis with numerous mucous and club cells. Also, *Seriola dorsalis* had four pairs of crescent-shaped gill arches with primary and secondary lamellae lined by chloride cells, a few mucous cells, columnar cells, and pavement cells. The liver of the *Seriola dumerili* fish had less obvious lobulation and consisted of hepatic cords separated from each other by blood sinusoids. Also, intracellular melanomacrophage aggregations, less typical portal triads, and intra-hepatic exocrine pancreatic tissue in the hepatic tissue were noticed.

Introduction

Seriola dumerili (yellowtail amberjack), under the family of Carangidae, was described as a highly mobile pelagic species. They have a preferred water temperature of 17-24°C [1]. It is known that young fish measuring up to 7 kg often form shoals. Usually, young Seriola dorsalis are spotted near the coast, whereas adult fish inhabit deep reefs. Juvenile yellowtail amberjack was not commonly seen; they were observed at a long distance from shore, often in relation to floating debris. Juveniles are distinguished by a yellow color with black bands that change when they mature and measure about 30 cm in length. Yellowtail with elongate fusiform body shape and a deep, forked yellow tail have other common names, including mossback, forktail, forktail, yeller, kingfish, jurel (Spanish for yellowtail), and yellowtail jack [2-3-4]. The common names of *Seriola dumerili* by vernacular (Arabic language) are Barema or Sholah in Libya [5] and Gazala or Hamam in Oman [6], and there was no official trade name. Also, *Seriola dumerili* is known by the vernacular (English language) in the United States (USA) as greater amberjack or rock salmon [7], and greater yellowtail in South Africa [8].

Historically, yellowtail worldwide have been lumped into a single species (*S. lalandi*); however, a study by [9] has found that yellowtail make up three distinct genetic populations worldwide. Yellowtail found in the Southern Hemisphere are still classified as *S. lalandi*, and the yellowtail found in the Northwestern Pacific are now *S. aureovittata*. The yellowtail found in California and the northeastern Pacific is now classified as S. dorsalis [9-4]. In aquatic animals, the skin works as a barrier to isolate the internal structure from the external environment and exhibits a significant role in protection, osmo-regulation, and sensory perception, as well as a protective barrier against external pathogens [10-11]. However, fish species have some variations in skin features regarding the existence or lack of scales [12]. The type, number, and size of scales serve as indicators of lifestyle character. Fish species that swim rapidly in fast-flowing water often have a large number of scales, like trout, whereas scaleless characteristics are observed in fish that prefer hiding in confined spaces like caves or crevices, like many catfish [13-14]. The epidermis, which forms the outer cellular layer of the integumentary system, arises from the ectoderm in the embryonic stage. The basic cellular structure is found in the epidermis of all fish; however, some variation in the cell types may be detected based on fish species [15-14]. The cellular components of the epidermis include epithelial cells, club cells, basal cells, and goblet cells, which secrete mucus substances to keep the skin surface wet and protect the body from environmental stresses [13-16]. The dermis comprises scales and collagen stroma, which together guard muscles and organs [17]. The dermis developed from the mesoderm and is composed of two strata: stratum spongiosum and stratum compactum [18]. The hypodermis is situated between the

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dermis and the muscles and contains lipid cells, blood vessels, and lymphatic vessels [19]. In addition to being the primary sites of respiration and gas exchange, gills have a secondary function related to feeding habits, where the arrangement and size of the gill rakers determine the size of food particles eaten by the fish [20-22] reported that long rakers are common in filter-feeding fish, whereas short rakers are distinctive to omnivores and carnivores. The liver plays an important role in regulating cholesterol levels in the body, as it not only controls the body's lipid content, such as lipoproteins, but also synthesizes bile acids and excretes excess cholesterol in the feces. In addition to its primary function in the metabolism of nutrients, it is and a reservoir for both fats and glycogen [23- 24]. Since then, fish have emerged as a model in toxicology [25], biomedicine [26], evolutionary ecology [27], and regeneration research [28].
The morphological and histological study on various fish species has become an extensive research area, and to our knowledge, there have been no studies on the natural morphology and histological structure of the *Seriola dumerili* (Barema or Sholah) fish in Libya. Therefore, the current research focused on studying the normal histological structure of the gills, skin, and liver of *Seriola dumerili* fish, which are used the laboratory fish models for studies in toxicology and biomedicine research and evolutionary ecology. Therefore, the study aimed to provide an overview of the tissue of the *Seriola dumerili* fish. Although the design of this study does not include all organs and tissues, it represents a comparative guide with other fish species. Furthermore, the data of the current study may be used in future histological studies and clinical diagnoses or for use in future scientific research.

Materials and Methods

The current work was conducted on live, healthy adult specimens (health of fish was detected based on assessment of the internal and external observations during autopsy) of Yellowtail Kingfish, *Seriola dumerili* (Family: Carangidae), Fig. (1). A total of twenty fish were collected twice (ten fishes each) from two fisheries sites on the eastern Libyan coast during one season at Sousse by the local fishermen. After dissection, a sample of skin, gills, and liver was removed and immediately fixed in Bouin's solution for 24 hours for histological and histochemical purposes. Fixated tissues were dehydrated in an improved series of alcohols. Tissues were processed and infiltrated with paraffin, it was filtered into xylene, and incorporated into paraffin wax (melting point between 56°C and 58°C.They were cut using a rotary microtome at 5 to 7 µm. The sections were fixed onto glass slides, deparaffinized, and they were stained with Harri's hematoxylin and eosin (H&E) to obtain the general histological structure, Periodic Acid Schiff (PAS), and Crossman's trichrome stain (histological techniques were applied in accordance with standard manual methods of [29]. The stained sections were mounted in Canada balsam and covered with a cover slide. Histological sections were examined by light microscope (Nikon Eclipse E400 with digital camera Nikon DS-Fi1), and histological structures were recognized and photographed. The body weight (gm), length, and width (cm) were measured.

Results

Morphological observations

The morphological study revealed that the male *Seriola dumerili* had a flat shape, a medium-sized body with an average weight of 566.3±82.5 gm, a yellowish color in the cranial and dorso-lateral region, and fusiform ends. Our result also revealed that the male *Seriola dumerili* had 25.7± 0.3 cm in length and 6.8± 0.2 cm in width (which represented **maximum vertical distance between dorsal and ventral margin of the fish body** depth).

Figure 1. A photograph of male Seriola dumerili fish showing yellowish color in the cranial and dorso-lateral regions and, medium-sized flat body.

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Examination of the *Seriola dumerili* fish skin revealed that the skin consisted of epidermis, dermis, and adipose tissue, which represent the hypodermis layer. The epidermis was found to be composed of basal columnar cells supported by a basement membrane and many layers of polyhedral keratinocytes with no superficial stratum corneum. Also, the epidermis contained surface mucous-secreting cells (goblet cells) and numerous club cells, which appeared spherical in shape with pale cytoplasm, and those cells were clearly marked by Periodic Acid Schiff's (PAS) stain and Crossman's trichrome stain. The club cells located deeper in the epidermis are interspersed between polyhedral keratinocytes. Also, many scales of various sizes and shapes covering the epidermal layer were seen. The skin of *Seriola dumerili* also showed differences in the epidermal thickness and cell allocation in various body areas. The dermis of *Seriola dumerili* consisted of thin, loose connective tissue layer (stratum spongiosum) containing collagen fibers with diffuse melanocytes and a thick, dense connective tissue layer (stratum compactum) consisting of abundant bundles of collagen fibers that run along the skin surface and are supported by the hypodermis of fat tissue. Surface mucous cells in the epidermis showed a positive reaction with the PAS stain.

The histological features of the skin of the *Seriola dumerili* fish from the Libyan eastern coast reflect their adaptations to their lifestyles and habitats and were found to consist of the epidermis, dermis, and hypodermis layers with scales of various sizes and shapes covering the epidermal layer. These findings are described in previous research in many species of fishes, such as common carp (*Cyprinus Carpio*) and catfish (*Silurus Triostegus*) by [10]and in polypteriform fish by [30]. [10] reported that the scales of the fish are specialized for protection against external threats, while fish that lack scales rely on basic skin structures for flexibility and sensory functions. [14-13] also reported that the type, number, and size of scales serve as indicators of the lifestyle character of fish. Our results also revealed the presence of goblet cells and numerous club cells in the epidermis of *Seriola dumerili* fish. Mucous and club cells have been morphologically identified as elongated or spherical cells containing pale acidophilic cytoplasm and basal nuclei [31- 32]. Also, reported that goblet cells have a heterogeneous distribution across the body, which indicates that specific skin structure and function adaptations vary according to different environmental and biological needs. [33] reported that the mucus cells exhibit a protective role against pathogens and facilitate movement, and [10] also added that the mucus cells form a mucosal layer, which plays two vital functions including protection and lubrication and observed that the club cells produce a chemical substance that disseminate in the water when the skin is injured to alert other fish from the same species to surrounding threat.

Differences in the number and distribution of mucus and club cells in the epidermis of the Indian common carp (*Cyprinus carpio*) and the other three major Indian carp species (*Catlacatla, Labeorohita*, and *Cirrhinamrigala*) were observed by [34]. They reported that an increase in club cells occurs as a result of a decreasing in mucus cells to enhance the defensive mechanism. The pre-epithelial barrier consists mainly of three parts, including cutaneous mucus, an epithelial barrier that varies in its structure according to species, and scattered lymphoid tissue associated with the skin [35]. It has been documented that any alteration in the biochemical and immunological composition of fish skin mucus exposes fish to more pathogens and winter syndrome. In the current study, differences in the epidermal thickness and cellular distribution in the skin of *Seriola dumerili* were noticed. However, the thickness and cell composition of the three layers are highly variable and depend on many internal and external factors, including life stage, sex, reproductive status, nutrition, and health status, among others [36- 37].

The histological structure of the dermis and hypodermis of *Seriola dumerili*, which is described in the present work, has been documented in catfish (*Silurus triostegus*) by [10] and in turbot fish (*Scophthalmus maximus*) by [38]. The cellular component in the epidermis contains epithelial cells, club cells, basal cells, and goblet cells, which secrete mucus substances to preserve the skin's moisture and protect the body from environmental stressors [13-16]. The dermis comprises scales and collagenous stroma that together guard muscles and organs [17].

Morphological observations of the gills revealed that the gills of *Seriola dumerili* consisted of four pairs of crescent-shaped arches arising from the floor to the roof of the buccal cavity (posterior edge). Each pair of the gill arches was supported on the anterior portions by convex cartilaginous gill rakers. Each gill arch bears several gill filaments or holobranchs (primary lamellae). Each primary lamella comprises secondary lamellae (hemibranchs) extending on the two sides and is free in the opercular cavity (Fig. 4A-C).

Primary lamellae, also known as gill filaments, are the initial branching structures extending from the gill arch. They are the sites where water flows across and where secondary lamellae are attached. These structures are crucial for gas exchange, as they maximize the surface area of gills, allowing fish to absorb oxygen into the blood and release carbon dioxide into the water.

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The gill arches (brachial arches) consist of a chain of ring bones that represent the supporting structure of gill filaments. Due to the feeding behavior of fish, sand, shell fragments, and other debris enter the mouth, causing damage to gill filaments and the cheek cavity used for ventilation. Gill rakers are essential structures that prevent debris from adhering to the gill filaments and thus reduce their surface.

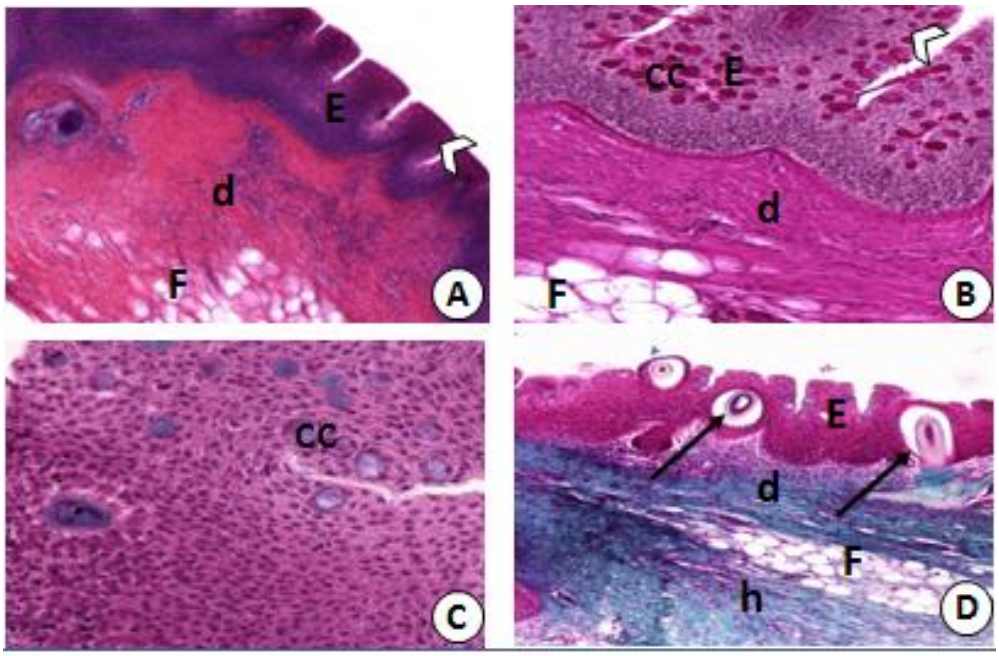

Figure (2A-D). Histological sections of male Seriola dumerili fish showing: A): epidermis (E), connective tissue in the dermis layer (d), and adipose tissue (f) (H&E, X200). B): numerous club cells (CC) and surface mucous cells (arrowhead) in the epidermis; note the positive reaction of surface mucous cells with PAS stain (PAS, X200). C): Marked staining of club cells (CC) in epidermis (Crossman's trichrome, X200). D): Many scales (arrows) arise from the epidermis, dermis, epidermis (E), dermis (d), and adipose tissue (f) (Crossman's trichrome (X200)).

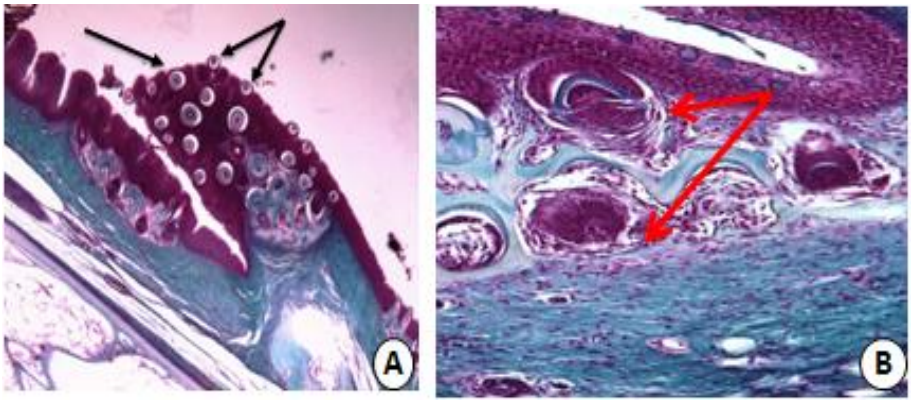

Figure (3A-B): Histological sections of male Seriola dumerili fish showing: A): Epidermis (E) with numerous scales (arrows), connective tissue of dermis (d). B: Some scales arise from the epidermis dermis (red arrows) (Crossmon's trichrome stain, A X100 and B X200).

The primary lamellae (gill filaments) are thin and long filament structures arranged in two alternating rows arising from the gill arch. The gill filaments are the principal location where the process of gas exchange occurs. As they increase the surface area of gill filaments. Several small blood vessels pass through the gill filaments known as capillaries, which are dark red in appearance. The tiered structure of gill filaments, along with constant water flow, ensures they do not adhere, thus increasing the surface area for gas exchange. Although fish is affected by the force of gravity, the presence of supporting structures is not necessary because the continuous water flow, which is the medium for gas exchange, provides support for gill filaments and maintains them separated. The secondary structure of gill filaments, covering the primary lamellae, immensely increases the surface area of gas exchange.

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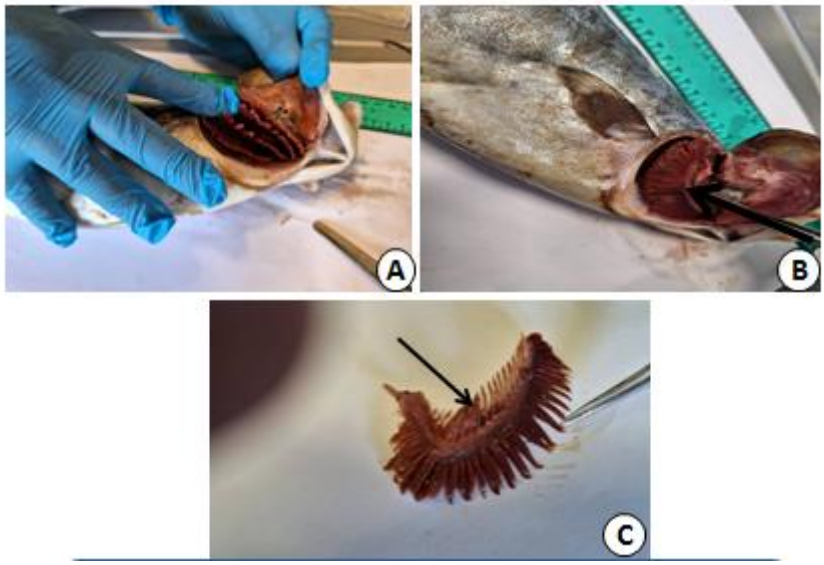
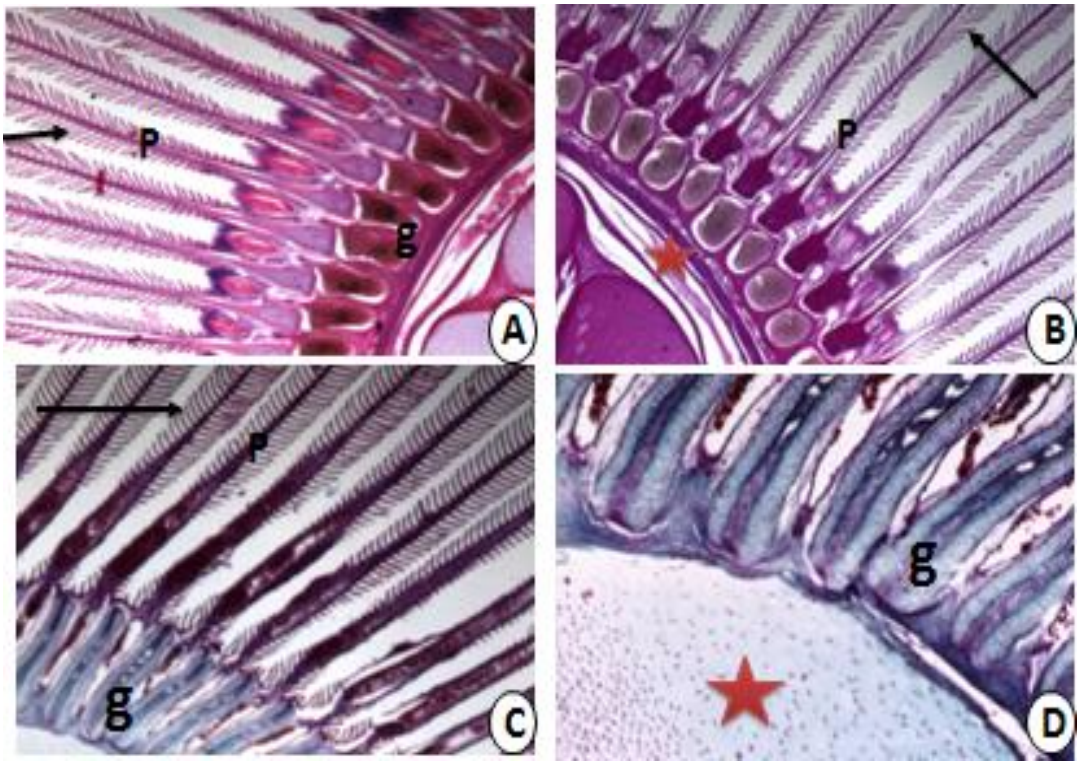


Figure(4A-C). Photographs of male Seriola dumerili fish showing four gill arches (thick arrow) with rows of primary lamellae extended from the posterior edge of gill arches, gill rakers from the anterior portions of gill arches (thin arrow), and gill cover (red arrow).

Examination of histological sections of the gills of *Seriola dumerili* showed that the gills consisted of gill arches bearing many primary lamellae supported by a gill raker. The gill arch extends to form a thin cartilaginous core that supports each primary lamella along with the connective tissue. Furthermore, these supporting structures of primary lamellae are recognized clearly by using Crossman's trichrome stain (Fig. 5A-D). Each primary lamella is well vascularized (mainly consisting of blood space capillaries; the afferent arterioles are found at the base of the secondary lamellae and efferent arterioles at the tips of the lamellae) and bears many secondary lamellae of variable length along both sides of the primary lamellae, which are arranged perpendicularly and covered by pavement cells, few mucous cells, pillar cells, and chloride cells (Figure 6).



Figure(5A-D) Histological sections of male Seriola dumerili fish showing the edge of gill arches (g) with primary lamellae (P) and secondary lamellae arising from each primary lamella (arrows) (A and B, H&E stain, X 200). C and D showing primary lamellae (P) and secondary lamellae arising from each primary lamella (arrow) supported by gill arches (g) and cartilage rakers (star) (Crossmon's trichrome stain, X200).

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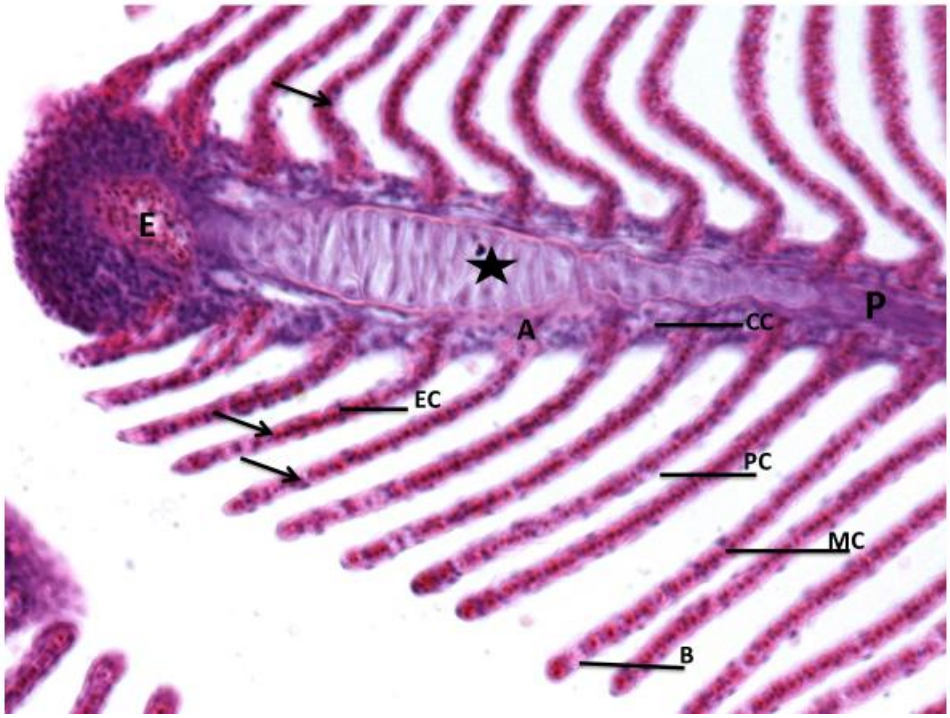

Figure6 Histological section of male Seriola dumerili fish showing primary lamellae (P) supported by cartilage (Star); secondary lamellae arise on each side of primary lamellae (Arrows) and consist of pillar cells (PC), pavement epithelial cells (EC), chloride cells (CC), blood capillaries (B), efferent arteriole (E), and afferent arterioles (A) and few mucous cells (MC) (MC)(H&E stain, X400).

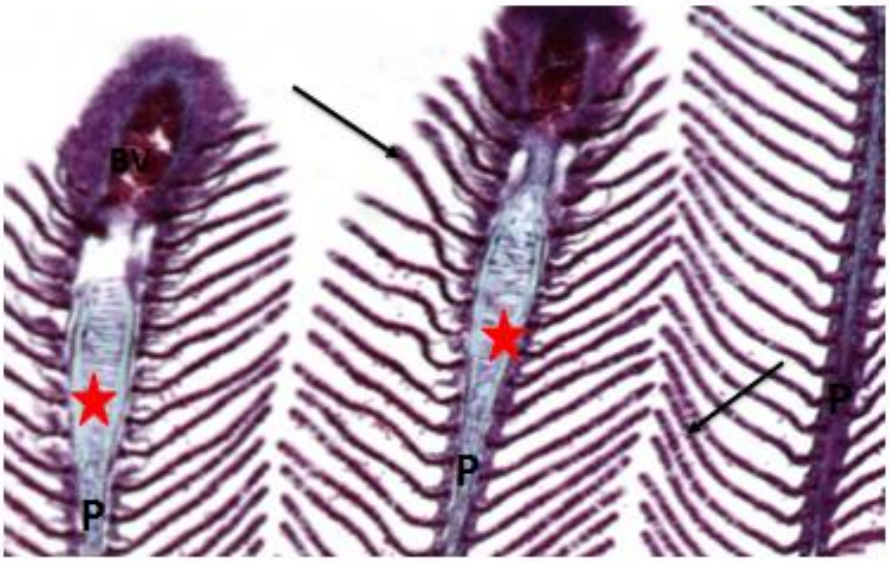

Figure 7. Histological section of male Seriola dumerili fish showing primary lamellae (P) supported by cartilage (stars), secondary lamellae from each primary lamellae (arrows), and blood vessels (BV) (Crossmon's trichrome stain, X400).

Our result revealed that the *Seriola dumerili* had four pairs of gill arches and showed a typical morphological and histological structural organization as in most other fish species [39- 40- 41- 42- 43- 44]. Conversely, [45] documented that the gill system of puffer fish (*Lagocephalus sceleratus*) consists of three pairs of gill arches, which may be related to significant adaptive modifications. However, other authors in catfish reported that the gills consist of five pairs. Furthermore, the fifth pair is under developed, and gill filaments are absent [40-46]. By contrast, [47] described in Sengal that the fifth pair is well-formed and possesses gill filaments. [48- 49] confirmed a clear link between the morphological adaptations of fish gills and their feeding pattern.

Our results of *Seriola dumerili* regarding the gills confirmed the findings of [44], they reported that the gill arches bear primary and secondary gill lamellae coated with a mucous epithelial layer and provided by a

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supporting structure of hyaline cartilage. The gill filaments are supplied by a small afferent artery that is a branch from the gill arteries passing along the longitudinal axis through the gill arches. Our observation revealed also that secondary lamellae are covered by pavement cells, a few mucous cells, pillar cells, and chloride cells that play primary roles regarding gas exchange, blood acid–base homeostasis, and ionic regulation [50-51- 45]. [44] reported that these cells function in the preservation of ion balance, the secretion of mucus, and the development of protective and functional layers.[45] described that the primary and secondary lamellae of puffer fish gills are interspersed with several mucous cells and chloride cells. The mucous cells serve as a lubricant to facilitate the unimpeded passage of food through the pharynx, in order to protect the epithelium from any mechanical injuries [52-53-54]. Furthermore, fish depend on mucous cells in their immune response against pathogens [55- 56]. [45] also highlight that the pillar cells have two main functions, including the contractile capacity and blood flow control. Chloride cells are integral to osmoregulation and acid-base regulation, and are also found in greater numbers and larger sizes in fish adapted to seawater [57- 58- 44].

[59] previously observed that chloride cells were located on the lamellar epithelium in freshwater fish: Oreochromis niloticus and Clariasgariepinus [60] in Lophiosilurus alexandri; and [61] in armoured catfish. It has been reported that ion regulation in freshwater fishes may be influenced by, chloride cells are found in greater numbers on the secondary lamellae [62- 63-64]. Whereas studies conducted by [65], [66-67] suggested that seawater fishes adapt to high salinity by increasing the number of chloride cells. Furthermore, the release of salt into a hypertonic environment may be attributed to the presence of chloride cells [68- 69].

Histological examination revealed that the liver of *Seriola dumerili* fish is composed of hepatic parenchyma with a little connective tissue, and the liver capsule consists of loose connective tissue with a single layer of simple squamous epithelium. The liver of the *Seriola dumerili* fish appeared with less obvious lobulation. Each hepatic lobule consisted of hepatic cords extending radially from the periphery to a central vein, and one or two of the hepatic cords were thicker compared to the others. The hepatic cords consisted of polyhedral hepatocytes that usually had one nucleus. The hepatic cords were separated from each other by blood sinusoids that opened into the central vein and were lined by Küpffer cells and endothelium cells. Completely isolated bile without blood vessels, enveloped by a thin sheath of connective tissue between the hepatocytes, could be seen. Also, intracellular melanomacrophage aggregations in the hepatic tissue were noticed. Typical portal triads between the hepatic lobules were less observed. Also, the pancreas of *Seriola dumerili* was found to be distributed throughout the body cavity surrounding different organs, particularly the stomach and intestine. Moreover, intrahepatic exocrine pancreatic tissue located in the visceral portion of liver parenchyma around the branches of blood vessels was seen (Figure10).

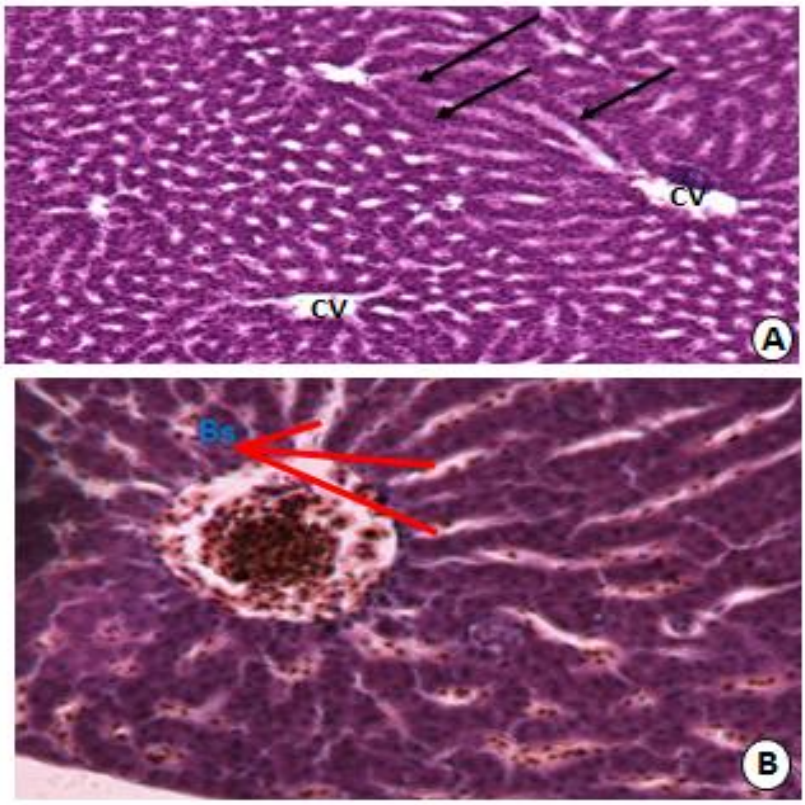

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Figure (8A-B). Histological sections of the liver of male Seriola dumerili fish with less obvious lobulation showing liver parenchyma consisting of hepatocyte cords (arrows) radiating from the central vein (CV) (H&E stain, X100); (B): blood sinusoid (Bs) (H&E stain, x400).

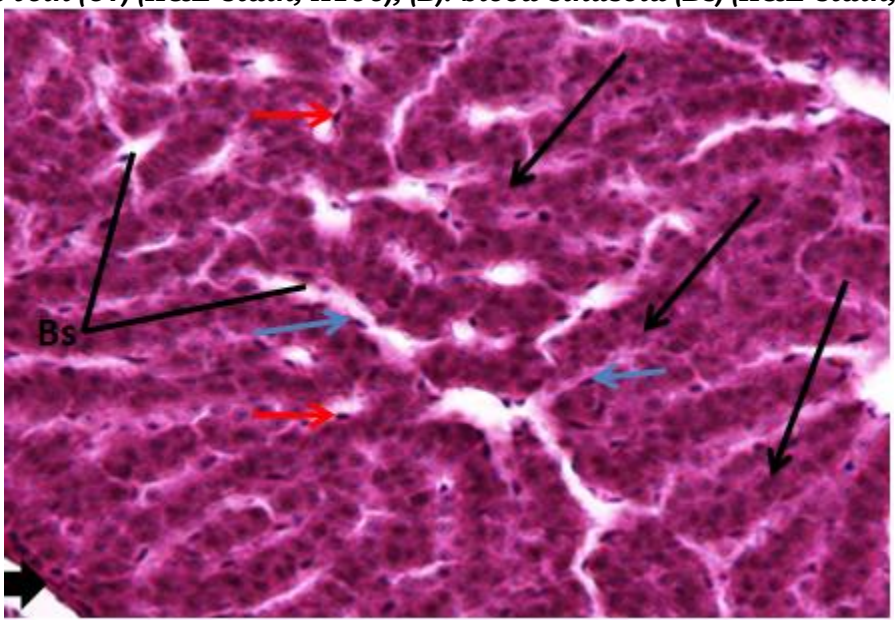

Figure9. Histological section of the liver of male Seriola dumerili fish liver parenchyma showing (A): Hepatic cords consisted of polyhedral hepatocytes with usually one nucleus (arrows), separated by blood sinusoid (BS), Küpffer cells (red arrows), endothelium cells (blue arrows), and capsule (arrowhead) (H&E stain, x400).

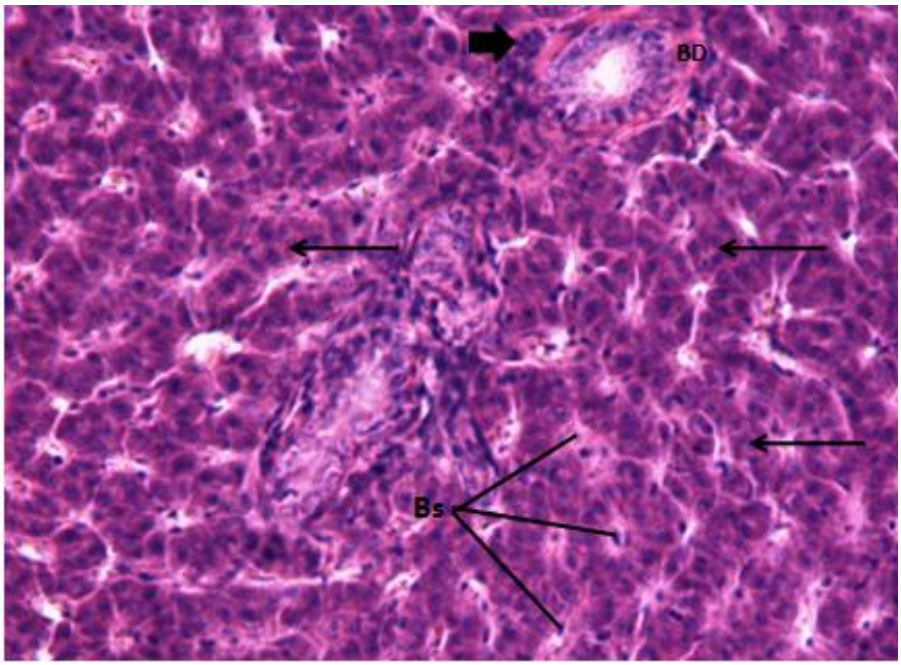

FigUre10 Histological section of liver of male Seriola dumerili fish showing liver parenchyma consisting of hepatic cords (arrows), blood sinusoid (Bs), isolated bile duct (BD), and melanomacrophage aggregations in hepatic parenchyma (arrowhead) (H&E stain, X 400).

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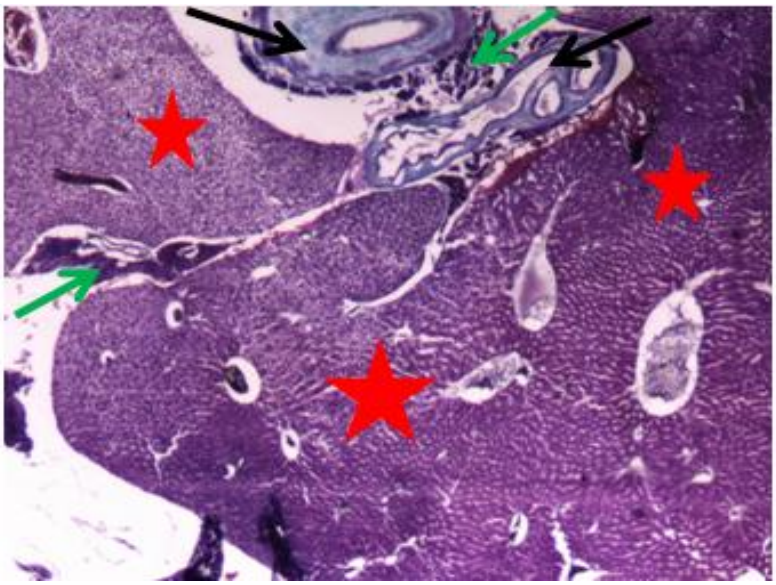

Figure 11. Histological section of the liver of male Seriola dumerili fish liver parenchyma showing hepatic tissue (stars), blood vessels (arrows), and diffused intrahepatic exocrine pancreatic tissue (green arrows) (H&E stain, x100).

Our findings regarding the liver structure were consistent with [70], who reported that the liver of the common carp was coated by a thin capsule with simple squamous and consisted of a continuous mass of polyhedral hepatocytes with centrally located nuclei arranged around irregular central veins associated with connective tissue surrounding the bile ducts and blood vessels, and the hepatocyte cords were separated by numerous hepatic sinusoids. Such findings were also documented by [71] in tigerfish, [72]. In pebbly fish, and [73]in common carp. However, the Kupffer cells that lined the hepatic sinusoid in the current study were not observed by [70] in the common carp [70]. Also, add that the veins in the common carp are not well organized and are disseminated within the liver parenchyma, and hepatic parenchyma or pancreatic tissue surrounds the veins, which occasionally coincide with an artery or a bile duct. In contrast to our result regarding portal triads, [70] documented the presence of portal triads in the liver of common carp. In the current study, less obvious liver lobulation in *Seriola dumerili* fish was in disagreement with [74], who reported that the liver of hagfish had well-developed hepatic lobules. Our findings support the report of [75], who reported that the structure of fish liver varies from the mammalian liver due to the unclear arrangement of hepatocytes into cords and lobules and the fact that typical portal triads are not evident. Differences in the biliary system in the fish compared to the mammals were documented by [76].

The current study found that the hepatic sinusoids of *Seriola dumerili* are lined by Küpffer cells and endothelial cells. The appearance of Kupffer cells in the liver tissue of fish is in agreement with [77] in the liver. On the contrary, [70] in the common carp, [78] in Scorpaena porcus, and [79] in zebrafish liver reported that the hepatic sinusoids had no Kupffer cells. Although hepatocytes, blood vessels, and bile ducts are found, the Fish liver tissue lacks lobules and portal triads, which represent the principal morphological structure in mammals [80]. Furthermore, the hepatopancreas can be apparent in various species; however, it has not been documented in higher vertebrates [72]. Our findings regarding the exocrine pancreatic acini in the liver tissue are in agreement with [70] in common carp and [81] in hake.

Our result regarding the exocrine pancreatic tissue of *Seriola dumerili* demonstrated that the pancreatic exocrine acini are of the intrahepatic type. A similar observation was also recorded by [82], who reported that the pancreatic tissue in *Oblada melanura* (Teleostei, Sparidae) was located in the visceral portion of the liver and originated from pancreatic exocrine acini, while the endocrine part was not observed in the liver tissue of this fish. Also, exocrine pancreatic tissue in Oreochromis niloticus and zebrafish was observed [79]. In contrast, the pancreas of *Ctenopharyngodon idella* has both diffuse and intrahepatic forms [83].

[84], who studied the structure of the digestive system in tropical freshwater fish, reported two types of fish livers: the first type contains pancreatic tissue, while the other type does not. Tropical freshwater fish often have livers with external pancreatic tissue called hepatopancreas[85].In the cardinal tetra, *Paracheirodon axelrodi*, reports indicate that the pancreas is generally disseminated within the fat and mesentery that link the intestine, stomach, and liver, forming a discrete organ. Our result regarding the bile duct was supported by [86] Teleost Livers. The structures of the biliary tract have been categorized based on the presence of concomitant blood vessels into four types: isolated type, the biliary-arteriolar tract type, the biliary-venous tract type, and the portal tract type. Researchers have also assumed that biliary tract structures were

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associated with feeding behavior and the adaptation of hepatic function, particularly regarding lipid metabolism.

Conclusion

We concluded that the morphological and histological features of the *Seriola dumerili* gills are adaptive modifications of the feeding habits and type of food.

Conflict of interest. Nil

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