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Research Article

Comparative Studies on the Histology of Cornea and Iris in Eyes of a Diurnal and a Nocturnal Red Sea Fish

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Abstract

This work aimed to investigate the comparative histological structure of cornea and iris in eyes of nocturnal fish, *Priacanthus hamrur* and diurnal fish, *Siganus rivulatus*. Samples of the selected fishes were obtained by hooks from the coral reef habitat at the northern Red Sea coast in Hurghada City, Red Sea Governorate. The collected fish samples were immediately dissected and their eyes were separated from the orbits, cut and immediately fixed in alcoholic Bouin`s fluid for 24 hours. Then, the routine histological procedures were carrid out to eye specimens and finally examined.

The histological results showed that cornea of nocturnal fish, *P. hamrur* is thicker than the diurnal fish, *S. rivulatus*. Cornea of *P. hamrur* consists of corneal epithelium having stratified squamous epithelial type; Bowman's layer; dermal stroma of loose connective tissue with un-striated smooth muscle fibers and contains some vacuoles in the pupil portion; scleral stroma is very thick, convoluted in the pupil portion and consists of compact collagenous muscle fibers; posteriorly covered by simple squamous epithelium (endothelium) that rests on Descemet's membrane. The Descemet's membrane is not detected in the cornea of *S. rivulatus* and the collagenous muscle fibers in the scleral stroma are arranged in a precise orthogonal array making a network. Scleral stroma is gradually transformed to the proper sclera containing hyaline cartilage and surrounded by the perichondrium.

Iris of nocturnal fish, *P. hamrur* has a thick matted layer of interdigitating melanocytes and fibroblasts. Its stroma is very thick and contains many blood vessels, fibrous connective tissue and richly vascularized. There are smooth muscles of sphincter pupillae and dilator pupillae muscles which extend along most of the iris. Iris of diurnal fish, *S. rivulatus* has a thin matted layer of melanocytes and fibroblasts. Its stroma is thick and contains many blood vessels, loose connective tissue. There are smooth muscles of sphincter pupillae which extend along most of the iris.

The present study concluded the histological structures of cornea and iris are varied between diurnal and nocturnal fishes to suite the change in the amount of light during day and night.

Keywords: *Priacanthus hamrur; Siganus rivulatus; Cornea; Iris; Nocturnal Red Sea Fish*

Introduction

The organizational layers of fish eyes can be distinguished into three different parts being the external, middle and inner layers [1]. The external layer is covered with the eye-ball as a sclera-corneal layer, which is classified into externally corneal stroma and internally sclera [1-3]. The middle or uveal layer consists mainly of the choroid, lens, ciliary body and iris. The choroid consists of a connective layer, lamina vasculosa and lamina choriocapillaris [4].

Collin and Collin [3] concluded that the cornea consists of an epithelium overlying a basement membrane. The corneal stroma with sutures and occasional cells have an anterior zone called Bowman's layer, although it is not well developed in some species and it does not exist in all bony fish. The third layer of cornea is the endothelium, underlined by a basement membrane known as Descemet's membrane whish separate it from the stroma [3-6].

The iris is the continuation of the choroid into the anterior compartment. The free margin of the iris defines the eye pupil. It mainly composed of connective tissue, blood vessels, melanocytes and smooth muscles at the posterior part [7]. Grynfeltt [8] recorded that there is a variety of muscles within the fish irises. It appears that many lower vertebrates have a sphincter muscle in the iris for constriction of the pupil or pupil dilation [9,10].

Vision has profound effects on the evolution of organisms by affecting survivorship through such behaviors as mating, foraging and predator avoidance [11-13]. Vision in fish depends on size and position of the eye, morphology and structure of retinal photoreceptors and structure of the pigment epithelium [14,15].

Fishes may be divided into two groups on the basis of their method of finding and procuring food; being diurnal and nocturnal predators. Vision in diurnal fishes plays the main role in capturing prey. Whereas, nocturnal fishes apply the senses of smell, touch and lateral line organ. Nocturnal fishes show photoreceptor adaptations to generally increase their sensitivity to light, while diurnal species show adaptations to increase color discrimination. However, that a switch from a diurnal to a nocturnal life style is indeed possible, it has been reported for several species [16-21].

As the cornea and iris control the opening of the eye pupil in a way that allows the entry of the required amount of light sufficient for vision. Also, as a result of the change in the amount of light between day and night, diurnal and nocturnal fishes must have different structures of cornea and iris that suits this role.

Aim of the Study

This work aimed to investigate the histological structure variations in the eye's cornea and iris between diurnal and nocturnal Red Sea fishes. The diurnal fish, *Siganus rivulatus* and the nocturnal fish, *Priacanthus hamrur* were selected to study this relationship between eye structures and dial activity.

Materials and Methods

Fish samples

A total of twenty fish samples {10 samples of rabbitfish, *Siganus rivulatus* (Forsskal, 1775) as a diurnal fish, and 10 samples of goggle-eye, *Priacanthus hamrur* (Forsskal, 1775) as a nocturnal fish} were collected by hooks from the coral reef habitats at the northern Red

Sea coast (with fishermen at Hurghada Fishing Port) in Hurghada City, Red Sea. Diurnal fish, *S. rivulatus* was fished during daytime, while nocturnal fish, *P. hamrur* was fished during night. All fish samples were adult specimens and freshly obtained.

The morphometric characters of the studied fish species were recorded for each fish. These morphometric measurements were standard length (SL); head length (HL) and eye diameter (ED).

Histological investigations

The collected fish specimens of the investigated fish species were immediately dissected and their eyes were separated from the orbits, cut and immediately fixed in alcoholic Bouin`s fluid for 24 hours. Then, they were dehydrated in ascending concentrations of ethyl alcohol, cleared in xylene and embedded in wax (M.P.: 58°C). Transverse sections were cut at 4 - 6µ in thickness; stained with Harris`s haematoxylin and eosin for routine histological examination. Finally, the slides were microscopically examined then photographed using a digital camera (Kodak, 14 megapixels) and then described.

Results

The morphometric measurements of fish eye showed that the eye of nocturnal fish, *Priacanthus hamrur* is very large, where the proportions of eye diameter (ED) to both head length (HL) and total body length (TL) were 37.3 + 2.76 and 10.8 + 0.77, respectively. On the other hand, the eye of diurnal fish, *Siganus rivulatus* is small, where the proportions of eye diameter (ED) to both head length (HL) and total body length (TL) were 21.6 + 2.88 and 4.04 + 0.38, respectively.

Generally, the fish eye is composed of three main layers; a sclera-corneal layer, a medial uveal layer and an inner retinal layer. Two chambers are included in the fish eye; an outer aqueous chamber bounded by the cornea and iris, and an Posterior vitreous chamber bounded by the iris and sclera (Figure 1 and 2).

Cornea and sclera

The cornea consists of two definite layers, a dermal stroma and a scleral stroma separated by a membranous stromal layer of connective tissue. The dermal stroma is continuous with the skin, whereas the scleral stroma is continuous with the sclera. The inside of the scleral stroma is covered with an endothelium (Figure 1 and 2).

The cornea of *Priacanthus hamrur* is thick and consists of many layers. The corneal epithelium is made of stratified squamous epithelial type. Bowman's layer consists of connective fibers under corneal epithelium. The dermal stroma is made of loose connective tissue with un-striated smooth muscle fibers and contains some vacuoles in the pupil portion. The scleral stroma is very thick and convoluted in the pupil portion. It consists of compact collagenous muscle fibers. The posterior surface of the scleral stroma is covered by simple squamous epithelium (endothelium) that rests on another layer of collagen and other extracellular material called Descemet's membrane (Figure 1A-1C). The histological examination showed that scleral stroma is gradually transformed to the proper sclera. The sclera consists of hyaline cartilage containing chondrocytes in its lacunae and externally surrounded by the perichondrium (Figure 1D-1F). At the corneoscleral junction, there is a limbus encircling the posterior endothelium and its thick underlying Descemet's membrane. Limbus has a meshwork of irregular channels lined by endothelium and supported by trabeculae of connective tissue (Figure 1A).

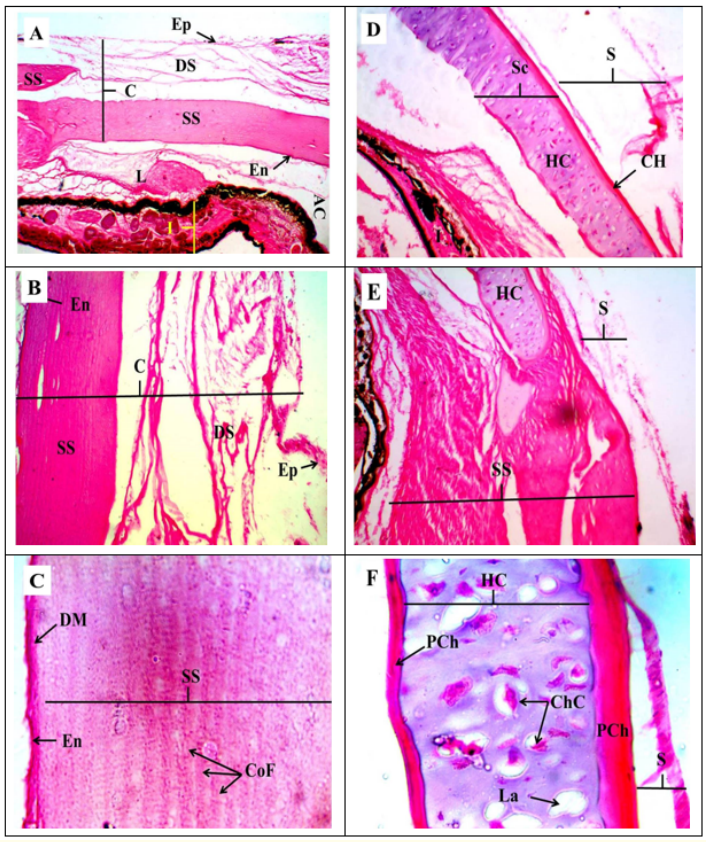

Figure 1: *Photomicrographs of histological vertical section in the eye of goggle-eye fish, Priacanthus hamrur showing: A, B) Structures of cornea showing corneal epithelium, dermal stroma, scleral stroma and endothelium layers. C) Magnification of scleral stroma. D and E) Showing transformation of cornea to sclera. F) Showing structure of sclera.*

AC: Anterior Chamber; C: Cornea; ChC: Chondrocyte; CoF: Collagen Fibers; DM: Descemet's Membrane; DS: Dermal Stroma; Ep: Epithelium; En: Endothelium; HC: Hyaline Cartilage; I: Iris; La: Lacuna; PCh; Perichondrium; S: Skin; SC: Sclera; SS: Scleral Stroma {H&E; A&D: x40; B&E: x100 and C&F: x400}.

The cornea of *Siganus rivulatus* is thin and it consists of many layers. The corneal epithelium is made of stratified squamous epithelial type. Bowman's layer consists of connective fibers under corneal epithelium. The dermal stroma is made of loose connective tissue with some un-striated muscle fibers. The scleral stroma is relatively thick and it consists of many collagenous muscle fibers arranged in a precise orthogonal array making a network. The posterior surface of the scleral stroma is covered by simple squamous epithelium or endothelium; the layer of Descemet's membrane is not detected (Figure 2A-2C). The scleral stroma is gradually transformed to the proper sclera. The sclera consists of hyaline cartilage containing chondrocytes in its lacunae and externally surrounded by the perichondrium (Figure 2D-2F).

At the corneoscleral junction, there is a limbus encircling the posterior endothelium. Limbus has a meshwork of irregular channels lined by endothelium and supported by trabeculae of connective tissue (Figure 2A).

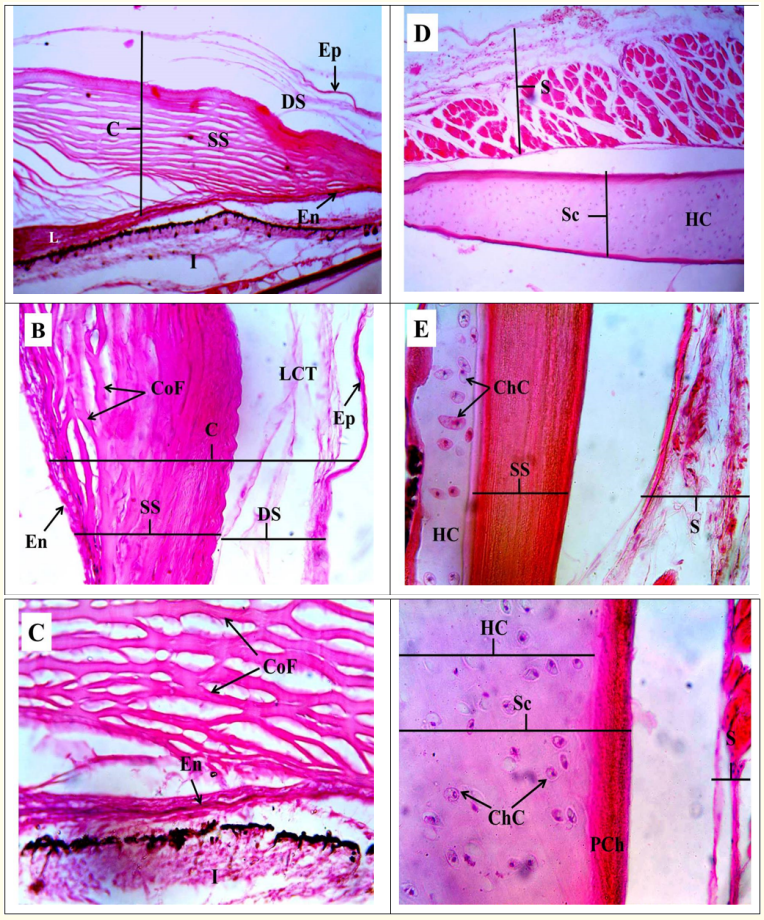

Figure 2: *Photomicrographs of histological vertical section in the eye of rabbit fish, Siganus rivulatus showing: A, B) Showing structures of cornea showing corneal epithelium, dermal stroma, scleral stroma and endothelium layers. C) Magnification of scleral stroma. D and E) Showing transformation of cornea to sclera. F) Structure of sclera.*

AC: Anterior Chamber; BV: Blood Vessels; C: Cornea; ChC: Chondrocyte; CoF: Collagen Fibers; DM: Descemet's Membrane; DS: Dermal Stroma; En: Endothelium; Ep: Epithelium; HC: Hyaline Cartilage; I: Iris; L: Limbus; La: Lacuna; PCh; Perichondrium; PE: Pigmented Epithelium; S: Skin; SC: Sclera; SS: Scleral Stroma {H&E; A&D: x40; B&E: x100 and C&F: x400}.

Iris

Iris is the extension of the choroid coat into the anterior compartment. Generally, the anterior surface of the iris is covered with an endothelium which is continuous with the endothelium covering the posterior surface of the cornea. This anterior smooth border of the iris was covered by a discontinuous combination of the pigment cell and connective tissue fibers. The spongy stroma in the middle of the iris comprised the reticular fibers of loose connective tissue, blood vessels and pigment cells. Posterior part of the iris composed of dense connective tissue, blood vessels and pigment cells. The posterior border of the iris was covered by a non-pigmented rough epithelium backed by a pigmented layer (Figure 3 and 4).

The histological examination of the nocturnal fish (*Priacanthus hamrur*) showed that the anterior surface of the central iris, near the pupil, is exposed to aqueous humor in the anterior chamber. It has a thick matted layer of interdigitating melanocytes and fibroblasts. The stroma of iris is very thick and contains some melanocytes, many blood vessels and fibrous connective tissue. This deep stroma is also richly vascularized and has different types of smooth muscles. In the posterior part of iridial stroma, there are the sphincter pupillae muscles in addition to the dilator pupillae muscles which extend along most of the iris. Together the two types of muscles control the diameter of the pupil (Figure 3).

The epithelium on the posterior side of the iris, adjoining the posterior chamber, consists of two layers of cuboidal cells. The external layer is the non-pigmented epithelium and the internal one is the pigmented epithelium, it is very rich with melanin granules. This epithelium of iris has many folds which increase its length (Figure 3).

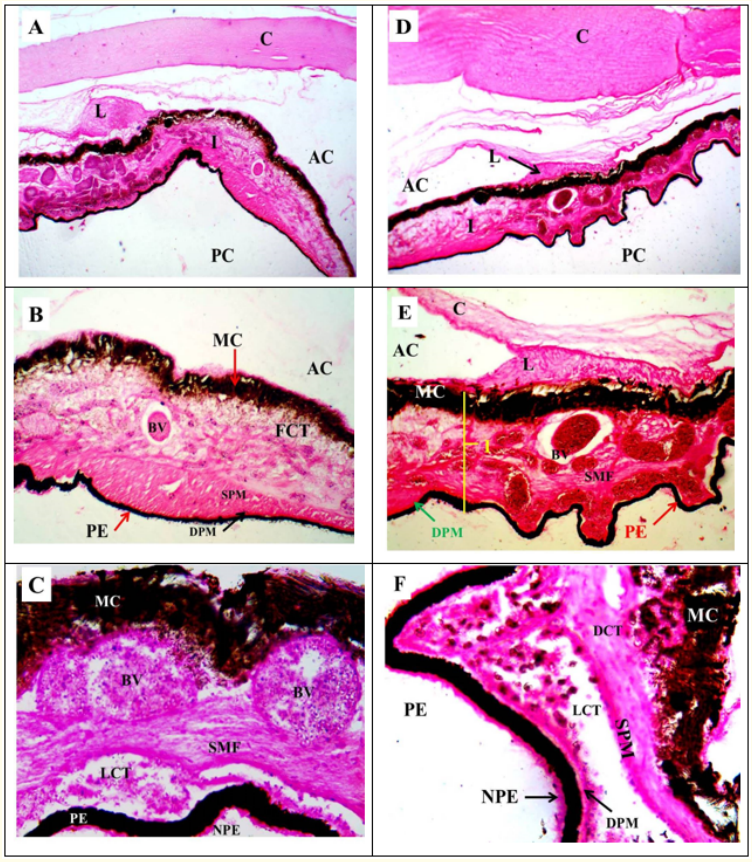

Figure 3: *Photomicrographs of histological vertical section in the eye of goggle-eye fish, P. hamrur showing: A, B and C) Structures of the right iris; D, E and F) Structures of the left iris.*

AC: Anterior chamber; BV: Blood vessels; C: Cornea; DCT: Dense connective tissue; DPM: Dilator pupillae muscles; FCT: Fibrous connective tissue; I: Iris; L: Limbus; LCT: Loose connective tissue; MC: Melanocytes; PC: Posterior chamber; PE: Pigmented epithelium; NPE: non pigmented epithelium; SPM: Sphincter pupillae muscles {H&E; A&D: x40; B&E: x400 and C&F: x400}.

The results of the diurnal fish (*Siganus rivulatus*) showed that the exposed surface of iris to aqueous humor has a thin layer containing melanocytes and fibroblasts. The underlying iridial stroma contains some melanocytes with varying amounts of melanin, blood vessels and fibrous connective tissue as well as loose connective tissue. In the middle stroma of iris, there are muscles of sphincter pupillae. The epithelium on the posterior iridial epithelium in *S. rivulatus* consists of the same components similar to that found in *P. hamrur*, but it is smooth without any folds (Figure 4).

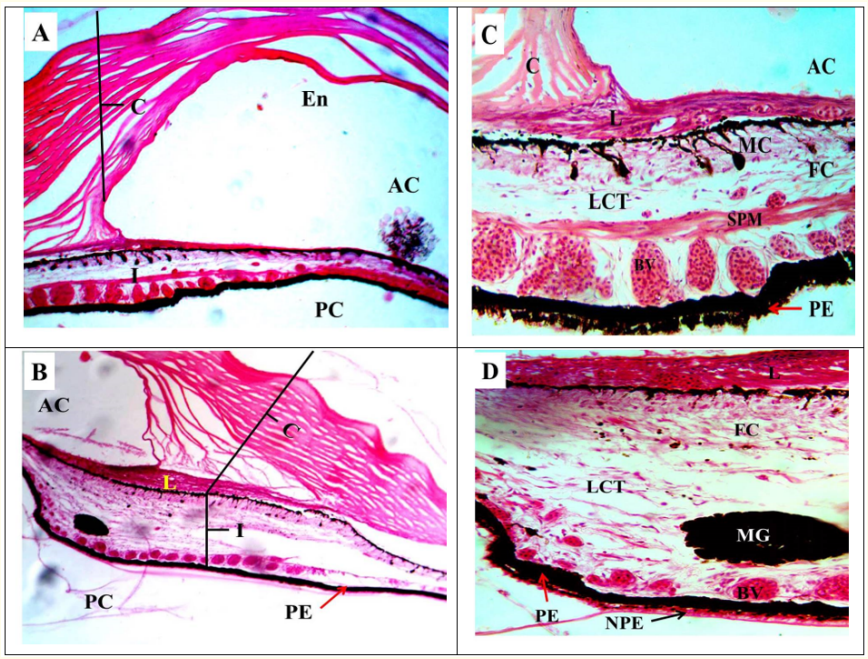

Figure 4: *Photomicrographs of histological vertical section in the eye of rabbit fish, S. rivulatus showing: A and C) Structures of the right iris; B and D) Structures of the left iris.*

AC: Anterior Chamber; BV: Blood Vessels; C: Cornea; Ep: Epithelium; FC: Fibrocytes; I: Iris; L: Limbus; LCT: Loose Connective Tissue; MC: Melanocytes; MG: Melanin Granules; PC: Posterior Chamber; PE: Pigmented Epithelium; NPE: Non Pigmented Epithelium; SPM: Sphincter Pupillae Muscles {H&E; A&B: x100 and C&D: x400}.

Discussion

In the present study, the nocturnal fish, *Priacanthus hamrur* has large eyes in relation to their head length (37.3 + 2.76). On the other hand, the diurnal fish, *Siganus rivulatus* has small eyes in relation to their head length (21.6 + 2.88). These results agree Darwish., *et al.* [22] who concluded that the nocturnal fish species have well developed eyes and retina compared with diurnal species. These results didn't agree Abdel Samei [23] who revealed that the studied nocturnal Nile fish species have smaller eyes than those studied diurnal Nile fish species. The present study recorded the presence of many layers in eye cornea of nocturnal fish, *Priacanthus hamrur* and the diurnal fish, *Siganus rivulatus*. The corneal epithelium; Bowman's layer; dermal stroma; scleral stroma and endothelium. This result related to that

recorded by Mansoori., *et al*. (2012) on *Siganus javus* eye and that reported by El Bakary [24] on *Mugil cephalus*. On the other hand, this finding not compatible with that reported by Collin and Collin [2] on pipefish, *Corythoichthys paxtoni* and salamander fish, *Lepidogalaxias salamandroides* and that reported by Senarat., *et al*. [4] on Stoliczkae's barb *Puntius stoliczkanus*.

The corneal epithelium is made of stratified squamous epithelium in both of investigated the nocturnal fish, *P. hamrur* and the diurnal fish, *S. rivulatus*. These results were disagree the results reported by Senarat., *et al*. [4] on the eye of *Puntius stoliczkanus* where its corneal epithelium is of simple squamous epithelial type; and Mansouri., *et al*. [6] on the eye of *Siganus javus* where their corneal epithelium is of stratified cuboidal epithelial type. On the other hand, Abdel Samei., *et al.* [23] recorded that the corneal epithelium is made of simple squamous epithelial type in *Orechromius niloticus*, *Bagrus bajad* and *Mormyrus kannume* and stratified squamous epithelial type in *Labeo niloticus* or stratified cuboidal epithelial type in *Clarias gariepinus* and *Chrysichthys auratus*.

Cornea of nocturnal fish, *P. hamrur* and diurnal fish, *S. rivulatus* consists of dermal and scleral components. The outer dermal stroma consisted of loose connective tissue with un-striated smooth muscle fibers and inner dense scleral stroma consisted of many layers of collagenous muscle fibers. These findings are in accordance with that recorded by El Bakary [24] on the eye cornea of grey mullet, *Mugil cephalus*.

The scleral stroma in eye cornea of nocturnal fish, *P. hamrur* is very thick and convoluted in the pupil portion where it consists of compact collagenous muscle fibers. But in eye cornea of diurnal fish (*S. rivulatus*), scleral stroma is less thick and consists of many collagenous muscle fibers arranged in a precise orthogonal array making a network. These results showed the adaptation of fish eyes inhabiting different dial activity to light. This result is in accordance with many authors suggested that light is one of the most important factors affecting on the vital processes of fishes [25-27].

The epithelium of iris in nocturnal fish, *P. hamrur* has many folds which increase its length; in addition to the great amount of sphincter pupillae muscles and dilator pupillae muscles in this fish iris which extend along most of the iris, all these characteristics control the diameter of the pupil to allow suitable amount of light to penetrate during the night activity. These results are in accordance with Sattari., *et al*. [28] who concluded that the presence of smooth muscles in fish iris can introduce the ability of pupil response to the light stimulation and pupillary constriction. They found that certain groups of bony fish display significant pupil mobility [29].

The present study showed that the iris in the studied fishes was covered by a discontinuous combination of the pigment cell and connective tissue fibers. The spongy stroma in the middle of the iris comprised the reticular fibers of loose connective tissue, blood vessels and pigment cells. Posterior part of the iris composed of dense connective tissue, blood vessels and pigment cells. The posterior border of the iris was covered by a non-pigmented rough epithelium backed by a pigmented layer. These results agree with those of Sattari., *et al*. [28] on the eye of *Siganus javus*.

Conclusion and Recommendation

The present study concluded that the histological structures of cornea and iris are varied between diurnal and nocturnal fishes to suite the change in the amount of light during day and night. These variations are represented in *P. hamrur* by the presence of Descemet's membrane in the scleral stroma and the presence of dilator pupillae muscles in deep stroma of iris as well as the great folding in the posterior epithelium of iris. These variations help this nocturnal fish to control the amount of light penetrating from the pupil to the eye retina. This study recommends conducting extensive studies on the eye retina as a facility of nocturnal fish adaption to see in the dark.

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