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Visual Adaptations of the Eye of *Mugil cephalus* (Flathead Mullet)

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Abstract: Vision in teleosts plays a great role in its vital processes in the different habitats that live in. Light, scanning and transmission electron microscopy were used to investigate the eyes of *Mugil cephalus.* Cornea of *Mugil cephalus* as one of shallow water teleosts has a special structural adaptations. Epithelial cells of the cornea are pentagonal with microplicae interwoven into microvilli. Concentric pattern over each epithelial cells are formed to increase the cell surface area and increase movement of oxygen and nutrients into the cornea. The pigment epithelium of the retina is thick and consists of simple columnar cells with melanin granules interspersed between them which helps in the protection of the rods external segments. Photoreceptors of the investigated diurnal species consist of numerous contracted cones and elongated rods. The findings of this work are discussed with respect to the photic habitat conditions of *Mugil cephalus* in the Mediterranean Sea.

Key words: Histology · Ultrastructure · Eyes · Teleosts

INTRODUCTION

Light is one of the most important factors affecting on the vital processes of fish [1-4]. Vision under water is limited by the optical properties of the water. In the Mediterranean Sea, almost 1% of the surface light reaches a depth of 255m [5].

The sclera is often composed of collagenous fibers and may possess ossified cartilage in some teleost species. The cornea of eye is a transparent window which mainly consists of an epithelium overlying a basement membrane. The density of its surface cells in bony fishes ranges from 2300 to 40800 cells /mm^2. The stroma with sutures and occasional cells has a modified anterior zone called Bowman's layer, although it is not well developed in some species and it does not exist in all bony fish [6].

Adaptations of the visual system allow fish to cope with the habitats specific photic environment. Several projections like microvilli or microplicae are found in the anterior corneal surface of humans, some species of monkeys and other mammals [7]. These microprojections increase the cell surface area and improve the absorbance of oxygen and nutrients and the movement of metabolic products across the outer cell membranes [8].

Differences in eye and retinal structure allow some fishes to exploit different habitats [9-11]. Fish that live in deepwater include larger eyes or a tapetum which reflects light back [12, 13]. Longer outer segments that increase the probability of photon capture or banked retinas [14], altering the absorptive properties of the visual pigments by amino acid alterations of visual pigment opsins that create visual pigments tuned to the visual tasks. The fish retina in the shallow water is rich in cone whereas rod-rich retinas are found in nocturnal and deepwater fishes [15].

Mugil cephalus (Flathead mullet) commonly known as the flathead grey mullet, can be found in tropical, subtropical and temperate rivers, estuaries and coastal waters. Flathead mullet are an important aquaculture species, particularly in many Asian and Mediterranean countries.

With the aid of light and electron microscopy, this study may provide us knowledge about visual structural adaptations of the eyes of *Mugil cephalus* in its habitat and represent preliminary study for other economically important teleost fish. Morphological, macroscopic and microscopic information about *Mugil cephalus* eyes can help in pathological evaluation of diseases and lesions. Histological and ultrastructural studies often play an important role to get these kinds of information.

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MATERIALS AND METHODS

Fish of *Mugil cephalus* where caught from Mediterranean Sea near Damietta. All of the fish were healthy and in good conditions without any external deformities. The fish were decapitated then their heads were immersed in 10% formalin solution. Sections of 6 micron thickness were prepared from eye through routine histological techniques and were stained with standard H&E. Histological study was done using light microscopy[4].

Scanning Electron Microscope Study: The cornea was fixed in 1% osmium tetroxide for 1 hour. Thereafter the specimen was dehydrated through graded series of ethanol and critical point dried. All specimens were mounted in Aluminum stubs covered with carbon tabs, sputtered with gold and observed under JEOL scanning electron microscopy (JSM-5300) at an accelerating voltage of 15kv in Electron Microscopy Unit in Alexandria University.

Transmission Electron Microscopy Study: The retina was cut into pieces, washed and postfixed in 1% osmium tetroxide for 2 hours at 4°C. The materials were dehydrated in ethyl alcohol to propylene oxide and embedded in araldite. Ultrathin sections were cut, stained with lead citrate and viewed under JEOL transmission electron microscopy in Electron Microscopy Unit in Alexandria University.

RESULTS

The epithelial cells of *Mugil cephalus* cornea are pentagonal possess microplicae interwoven into several microvilli. Numerous microridges like microplicae are a complex series of cellular projections but are significantly longer forming intricate and concentric patterns over each epithelial cell (Fig. 1a,b).

Cornea of *Mugil cephalus* consists of dermal and scleral components. The outer dermal portion composed of three layers; an anterior stratified cuboidal epithelium, bowman layer consisted of connective bundles, thick dermal stroma consisted of layers of fibers with stromal interfibrillar space and stromal swelling (Fig. 1c). The apical ends of the pigment cells of the retina are pentagonal in its shape (Fig. 1d).

The retina is consists of several layers, which from the outer to the inner are as follow: pigment epithelium, layer of rods and cones, outer limiting membrane, outer nuclear layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer, nerve fiber layer and inner limiting membrane (Fig. 2 a-b).

The pigment epithelium is thick consists of columnar epithelial cells containing melanin these cells send digitiform processes between photoreceptor cells. Layers of cones and rods which lies just below the pigmented epithelium. The rod cells are long with a basal nucleus. The cones are of the same structure as the rods. The outer nuclear layer represents the nuclei of the photoreceptor cells. The nuclei of the rods lie closer to the internal retinal layer. The inner nuclear layer contains the nuclei of several types of neurons. They form a thick middle layer of the retina which transmits impulses from the outer photo receptors to the inner ganglionic neurons. The outer and the inner plexiform layers form synaptic relationships between photoreceptors, bibolar and horizontal cells as well as between bibolar and ganglionic cells. Ganglion cell layer forms of a single row of neurons. Nerve fiber layer represents the axons of the cell of the overlying ganglionic layer which forms a single row. Inner limiting membrane is very thin and acts as a basement membrane. It represents a vascular layer interposed between the axons of the ganglionic cells and the vitreous humour of the eye ball (Fig. 2b,c).

The rods are thin and long with uniform shape and size. The outer segments of it are slender and cylindrical structures that reach the pigment epithelial cells and surrounded by its extensions. The ultrastructure of rods outer segment is similar to that of cones (Fig. 2d). The discs are surrounded by a double membrane. Most of them are at the same diameter of the outer segment and a few of them is short. The inner segments are rich in mitochondria found close to the stalk (Fig. 2e). Outer nuclear layer (ONL) occupied by nuclei of cones (con) and rods (rn). These nuclei adhere to the sclera side of the outer limiting (Fig. 2f) membrane which are differ in their form in rods the nuclei are elongated and opaque while that of cones are oval and granulated (Fig. 2f).

DISCUSSION

Mugil cephalus cornea has numerous mcrovilli, microprojection and microridges. The role of microprojection in aquatic vertebrates is uncertain. They increase the cell surface area and may improve the movement of oxygen and nutrients into the cornea and waste products and water out of the cornea. The presence of dissolved salts & the need to maintain an appropriate level of dehydration necessitates high epithelial cell densities to ensure a clear cornea [16].

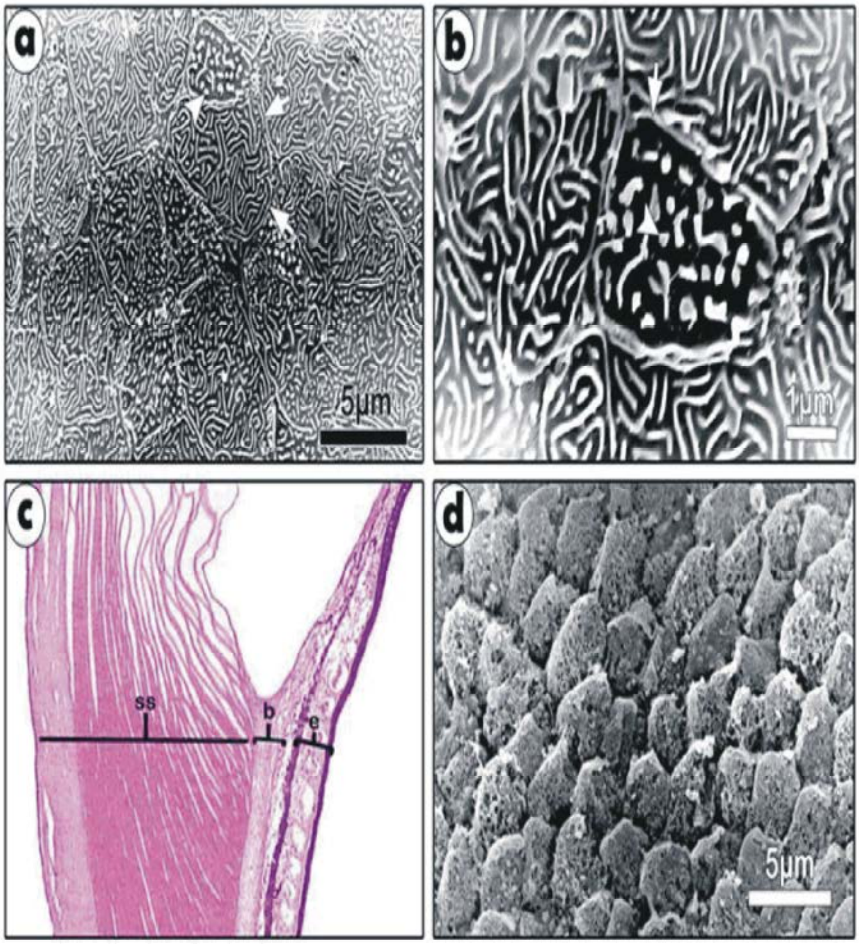

Fig. 1:
a-Scanning electron micrographs of the cornea pf *Mugil cephalus* showing the pentagonal cells (arrow) with microridges and projection
b-Enlarged scanning electron micrograph oe the cornea of *Mugil cephalus* showing microplicae (arrow) and microvilli (arrow head)
c-Light photomicrograph of the *Mugil cephalus* showing bowmans layer (b) between epithelium (e) and scleral stroma (ss)
d-Scanning electron micrograph of the apical ends of pigment cells of the retinal epithelium

The anterior corneal surface of *Mugil cephalus* is covered with a complex fingerprint like pattern of long parallel microridges. Ratfish, the deep-sea black shark and Australian lungfish possess microvilli over its corneal surface. The rat fish and the deep sea black shark are deep dwellers and exposed to low water velocities so its eye are adapted to its habitat and increase the surface area for metabolic exchange [16].

Microplicae do not provide surface area for osmotic exchange and may not stabilize the tear film as microvilli or microridges but providing ubiquitous support for the corneal coating with some increase in the degree of osmotic exchange.

Microvilli and microplicae in the epithelial covering of the cornea of *Mugil cephalus* may function to anchor the lubricating and crushing layer of mucus to protect the underlying plasma lemma from abrasion.

In the present study of the eye of *Mugil cephalus*, microridges found over the corneas exposed to marine environment. The freshwater fishes, *Lepidogalaxias salamandroides* [17] and *Lepisosteus platyrhinchus* possess microridges although the latter possess microplicae. This microprojection type may be not a characteristic for this environment.

Well-developed microridges occur only in teleosts inhabiting high osmolarity environments, e.g., marine or

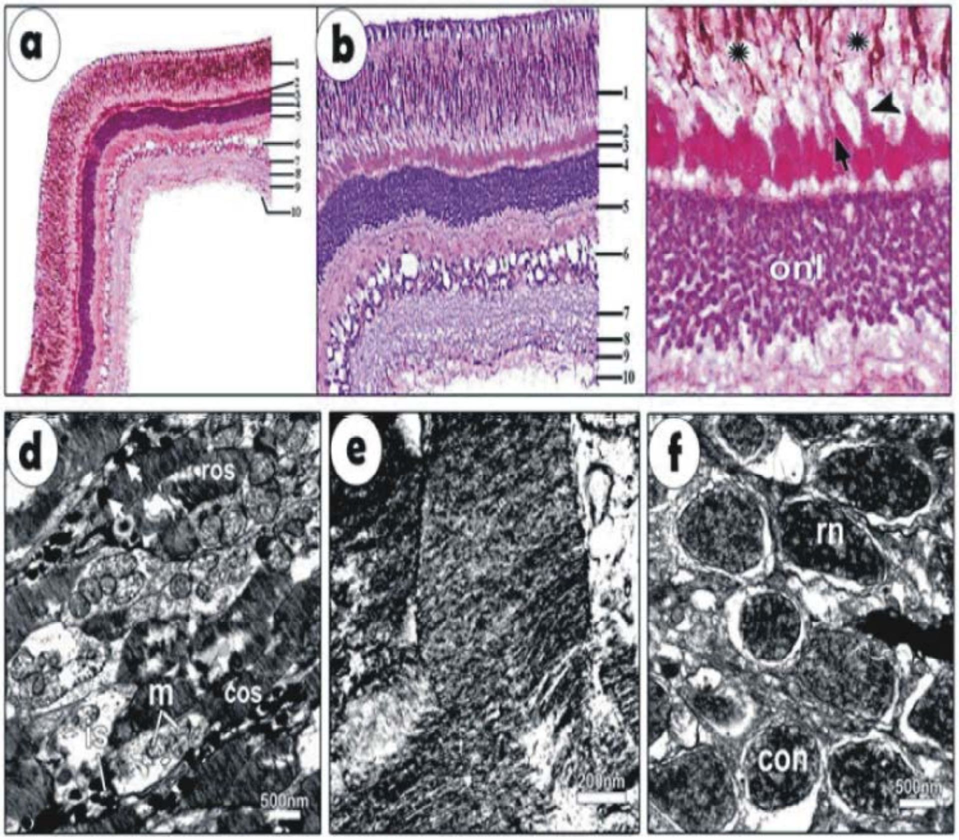

Fig. 2: Light photomicrograph of shows the organization of the retina of *Mugil ephalus*.
(1) pigment epithelium, (2) photoreceptor layer, (3) outerlimiting membrane, (4) outer nuclear layer, (5) outer plexiform layer, (6) inner nuclear layer, (7) inner plexiform layer, (8) ganglion cell layer, (9) layer of gabglion cell axons, (10) inner limiting membrance
b-Enlarged light photomicrograph of the retina of *Mugil cephalus*.
C-Detail of the photomicrograph. Single cones (head arrow), double cones (arrow), pigment epithelium cell extension (star), outer nuclear layer (onl).
D-Transmissin electron micrograph of the photoreceptors of *Mugil cephalus* showing pigment grains (arrows) dispersed between cones and rods outer (cos, ros) and inner segments (is), m: mitochondria.
E-Detail of the membrane discs of the outer segment of cones and rods.
F-Outer limiting membrane of the retina of *Mugil cephalus* showing rod (rn) and cone (con) nucleus

estuarine habitat. The salamander fish, *Lepidogalaxias salamandroides* live in freshwater ponds and have microridges with high tannin content and may be devoid of water [18]. The other freshwater teleosts all have microvilli and microplicae.

Diurnal teleosts light adapted retina differ from nocturnal species by contracted cones & elongated rods and a thick pigment epithelium like *Mugil cephalus* retina forming long extending melanin granules processes [19-21].

In the aestivating salamandroides, a new photoreceptor mosaic consisting of both cones and rods in the light adapted state were described [17]. The double cones optimize the perception of prey whereas the rods may increase the sensitivity of the visual system in the turbid environment in which this species lives. The results of the present work reveal the visual adaptations of *Mugil cephalus* to a thick pigmented epithelium that constitutes about one third of the retina thickness this help in the protection of the rods external segments this is similar to *Salmo gairdneri* [22] corroborating the importance of vision for teleosts.

The retinal pigment epithelium are optimally exposed to incoming light in the dark in the case of rods and under light conditions in the case of cones. This capacity might be important to enable the fish to find their prey under light and dark conditions or even when weather changes, resulting in sudden changes in light conditions. The high density of cones suggests that it has a relatively good photopic visual activity and may indicate diurnal activity [23].

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