

Visual adaptations of the eye of the gilthead sea bream (*Sparus aurata*)

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Abstract The current investigation represents the morphological description of the adaptation of gilthead sea bream *Sparus aurata* to its environmental conditions. For the achievement of this study twelve mature fishes were used for gross, light and electron microscope examinations. The cornea consists of three layers; an anterior stratified cuboidal epithelium, bowman layer, and a dermal stroma. A mucoid layer located between the dermal stroma and the anterior part of the scleral cornea, while the iridescent layer located between the anterior and the posterior scleral stroma. The retina is composed of ten layers. There are two areas of the retina; non-nervous and nervous. The non-nervous area is represented only by the thick pigmented epithelium layer. The retina has both rod and cone photoreceptors. The cones are differentiated into three types; single, double and triple cones. Rods inner segments contain numerous mitochondria while that of the cones has ellipsosomes. These ellipsosomes may function in condensing light in the outer segment. There is a clear correlation between ellipsosomes formation in the inner segment of cone and night condition.

Keywords Morphology · Eye · Retina · Cornea · Gilthead sea bream · TEM

Introduction

The gilthead sea bream (*Sparus aurata*) is a fish of the bream family *Sparidae* found in the Mediterranean Sea inhabiting sea grass beds, rocky and sandy bottoms as well as in the surface zone commonly to depths of about 30 m, but the adults may occur to 150 m depth (Abecasis et al. 2008). Furthermore, the gilthead sea bream (*Sparus aurata*) has an economical importance in the Egyptian coasts of Mediterranean Sea and the Bardawil fisheries.

Light and vision in the teleost fish are the most important environmental factors affecting on many the vital activities of fishes as; reproduction, migrations, swimming activities, searching for food (De Busserolles et al. 2013; Schmitz and Wainwright 2011). Teleosts fishes are the most common and highly distributed vertebrae in all types of waters of different qualities and at different depths, from the shallow well illuminated light zone to the deepest low illumination or completely dark zone.

Generally, the eye morphology reflects a general lifestyle of all vertebrates (Ebrey and Koutalos 2001), the different published articles on the fish eye reveal a high degree of diversity in their histomorphological and cytological characters, especially of the light sensitive retina according to the environmental conditions (El Bakary 2014; Fishelson et al. 2012; Reckel et al. 2002). Many morphological studies noted that the retina is highly adapted to have a good vision in the all types of waters (Heb 2009), in which other studies observed that there are many fishes were adapted to seeing in the deepest and darkest part of the sea, while there are a special group of fishes were adapted to seeing in the air (Nicol 1989).

From morphological published data about the fish eye, the diurnal fish species spending their life near the water surface have a well-developed retina, meanwhile the species of the fish spending their life in the deepest regions are often limited

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to one or two spectral classes of cones (Ali 1975; Donatti and Fanta 1999).

The main important aim of the current study is to demonstrate the correlations between the fish feeding habits, environmental living conditions and the morphological characters of the eye. With the aid of the light and the electron microscopy this study may provide us important knowledge about the visual structural adaptations of the eyes of *Sparus aurata* in its habitat and represent preliminary study for other economically important teleost fish. Morphological, macroscopic and microscopic information about *Sparus aurata* eyes can help in pathological evaluation of diseases and lesions. Histological and ultrastructure studies often play an important role to get these kinds of information.

Materials and methods

Samples

Twelve mature Fish of the gilthead sea bream (*Sparus aurata*) were collected after catching from the Mediterranean Sea in Damietta city, Egypt and transported in plastic aquariums to our lab within 2 h to carry out the gross morphology, light microscopic and scanning electron microscopic examinations. All collected fishes were healthy and in good conditions without any external deformities.

For gross morphological study

Five mature fishes were used to clarify the gross morphological features. The eye was photographed by digital camera (Cannon IXY 325, Japan).

For light microscopy

Four eyes were used in histological studies. The fish were decapitated then their heads were immersed in 10% formalin solution. Small specimens from the eye were taken in the fresh state. The samples were fixed in 10% normal buffer formalin. After 24 h, the samples were extensively transferred to 70% alcohol. The tissue samples were then dehydrated in ascending graded series of ethanol, cleared in xylene and impregnated and embedded in paraffin wax. Sections of 5–7 μm were cut using Leica rotatory microtome (RM 2035) and mounted on glass slides. Paraffin sections were used for conventional staining (H&E). The histological techniques and stains were adopted according to (Suvama et al. 2013).

Transmission electron microscope study

Three eyes were used in histological studies. The retina was cut into pieces of 1 mm^3 . The samples were immediately fixed

in a 6% solution of phosphate-buffered glutaraldehyde, pH 7.4, at 4 °C for 6 h (McDowell and Trump 1976). After initial fixation, tissues were washed in several changes of cold (4 °C) 0.1 M phosphate buffer every 15 min for 2 h. samples were then rapidly dehydrated through increasing concentrations of ethanol, transferred to propylene oxide and placed overnight in a 1:1 mixture of propylene oxide and epoxy araldite (Hayat 1986). Semi-thin sections (1 μm) were first cut and stained with toluidine blue and viewed with light microscopy. Ultrathin sections (60–100 μm) were then cut by a glass knife with an L.K.B. microtome and stained with uranyl acetate followed by lead citrate (Hayat 1986). The ultrathin sections were examined with a Jeol transmission electron microscope operating at 100 Kv. Faculty of Science, Alexandria University.

Results

Macroscopically, the large round sized eyes in *Sparus aurata* were found dorsolaterally on the rostral part of the head (Fig. 1a).

The external (sclera-corneal) layer of the eye consisted of the posteriorly situated sclera and anterior situated cornea. The

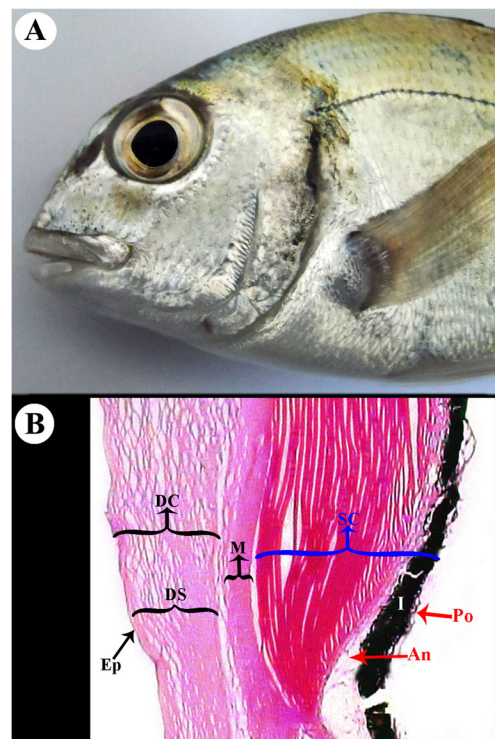


Fig. 1 View (a): represent the lateral view of the gilthead sea bream (*Sparus aurata*) while View (b) represent the Light photomicrograph of the cornea (C) to demonstrate; DC- dermal Cornea (epithelium + dermal stroma), EP- epithelium, DS- dermal stroma, M- mucoïd layer, SC- scleral cornea, I- Iridescent layer, Po- posterior scleral stroma, An- anterior scleral stroma

cornea is the transparent part of the external layer and consisted of three layers; an anterior stratified cuboidal epithelium layer, the bowman layer which formed from connective bundles, and a thick dermal stroma which consisted of layers of fibers with stromal interfibrillar space and stromal swelling (Fig. 1b). The sclera is white in color and consisted of a thick connective tissue layer. There is a mucoid layer located between the dermal stroma and the anterior part of the scleral cornea (Fig. 1b/DS, M and SC), in which the mucoid layer consists of the loose connective tissue with the small bundles of the collagen fibers and thin cells. Furthermore, there is a layer named iridescent layer located between the anterior and the posterior scleral stroma (Fig. 1b/I, Po and An) and this layer is thin at the center while thick peripherally.

The retina is the photosensitive (inner sensory) layer of the eye, which communicated by the optic nerve to the brain. The morphological structure of the retina in the examined fish is the same to that of other fishes. The retina is composed of ten layers which arranged from the outer to the inner in the following sequence; pigmented epithelium, photoreceptor 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).

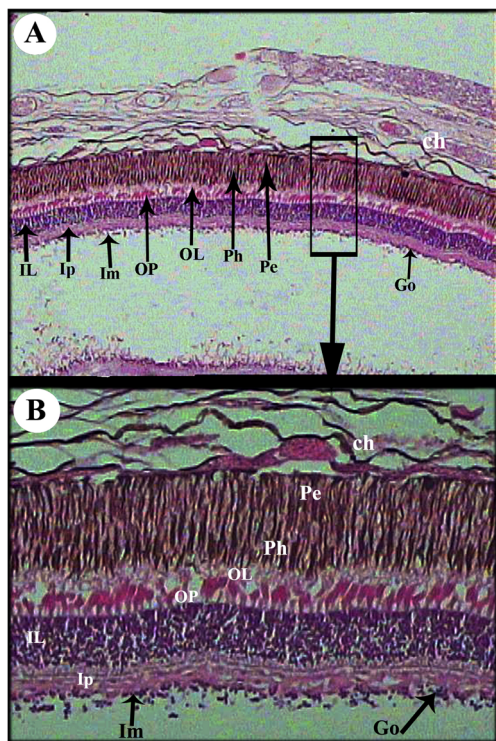


Fig. 2 Light photomicrographs of a transverse section of the retina of the gilthead sea bream (*Sparus aurata*) to show (View **b** is enlargement photo to the black demarcated area in the View **a**): im- inner limiting membrane; Ip- inner plexiform layer; IL- inner nuclear layer; OP- outer pexiform layer; OL- outer nuclear layer; Ph- photoreceptor cell; Pe: pigment epithelium; Go- ganglionic layer; ch- choroid plexus

There are two areas of the photosensitive retina; non-nervous and nervous area, in addition to the inner limiting membrane. The non-nervous layer is represented only by the pigmented epithelium layer of the retina. This thick pigmented epithelium layer consisted of a columnar epithelial cells containing melanin, which absorb the light passing through the photoreceptor layer of rods and cones (Fig. 2).

The nervous area of the photosensitive retina consisted of the following layers; photoreceptor layer of rods and cones, outer limiting membrane, outer and inner nuclear layer, outer and inner plexiform layer, ganglion cell layer, nerve fiber layer and inner limiting membrane (Fig. 2).

There are two types of the photoreceptor cells; rods and cones which situated under the thick pigmented epithelium layer. The retina of *Sparus aurata* possesses both rod and cone photoreceptors. The cones are differentiated into several types; single, double and triple cones (Fig. 3). The cones of the investigated species are structures consisted of outer and inner segments. The outer segments extended towards the pigmented epithelia (Fig. 4a). The inner segments of cone were characterized by ellipsosomes which are mitochondria with disintegrated cristae. Ellipsosomes are numerous, dispersed in the apical and basal part of the inner segment and contains a condensed pigment (Fig. 4a).

The rods are thin and long with uniform shape and size. The inner segment of the rods contains numerous large round mitochondria condensed in the apical part of the inner segment of the rods. The mitochondria of the rod cells are normal in appearance and do not exhibit any degenerative changes (Fig. 4b). Ultrastructurally, the outer segment of the rods is slender and similar to that of the cones and reaches the pigment epithelial cells and surrounded by its extensions. The discs of the outer segment are surrounded by a double membrane (Fig. 4c). Most of them are at the same diameter of the outer segment and a few of them is shorter.

The outer nuclear layer was occupied by the nuclei of the photoreceptor cells; cones (CON) and rods (RN). These nuclei

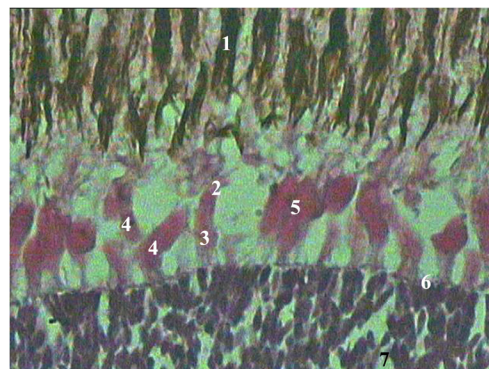
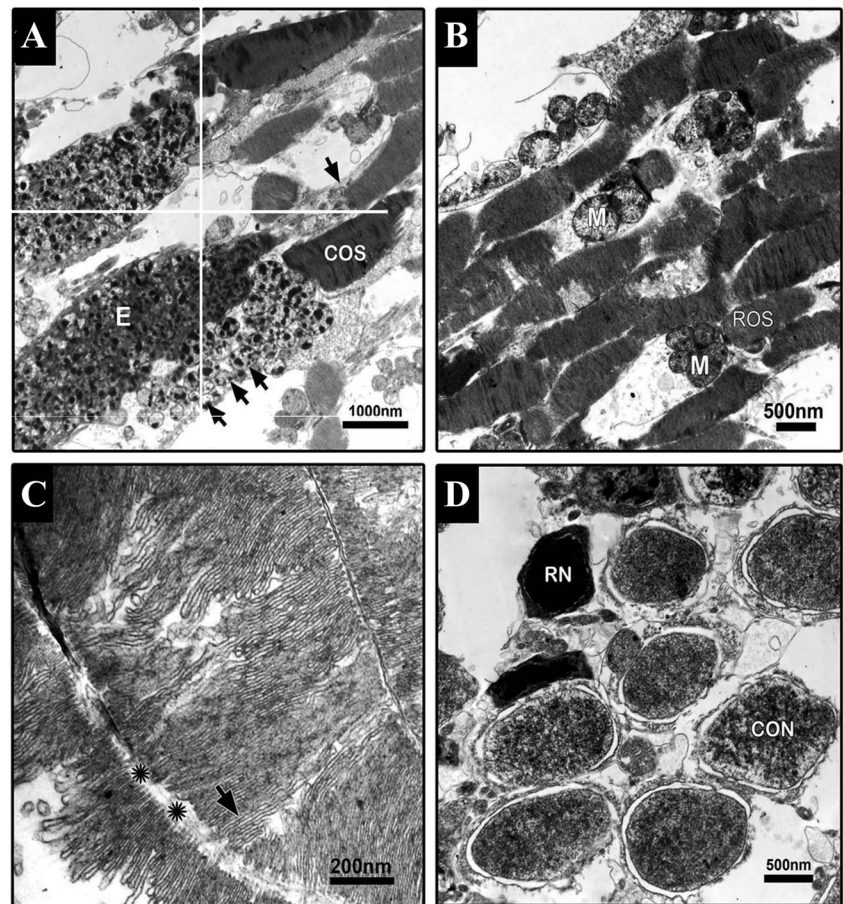


Fig. 3 Light photomicrograph of a transverse section of the retina layer of the gilthead sea bream (*Sparus aurata*) to show: 1- Pigment epithelium cell extensions; 2- outer segment of cones; 3- single long cone; 4- double cone; 5- triple cone; 6- outer limiting membrane; 7- outer plexiform layer

Fig. 4 View **a**: Transmission electron micrograph of the outer segments of cones (oc) to show: cones ellipsoids (e) with subsurface cisternae (arrows). View **b**: Transmission electron micrograph of the outer segment of rods (ROS) and the inner segments with circular mitochondria (M). View **c**: Enlarged transmission electron micrograph of the outer segment of photoreceptors showing the connection between neighbor cells (star) and the double membrane (small arrow). View **d**: Transmission electron micrograph of the outer nuclear layer of retina showing cone (CON) and rod (RN) nuclei



adhere to the sclera side of the outer limiting. The rod nuclei are elongated and opaque while, that of the cones is oval and granulated (Fig. 4d). The cone nuclei were arranged in a single layer, whereas rod nuclei were located in two or three layers. This layer of rod and cone nuclei results in a thick outer nuclear layer.

The inner nuclear layer considered as a thick middle layer of the retina to transmit impulses from the outer photoreceptors to the inner ganglionic neurons. The inner limiting membrane was very thin and acts as a basement membrane and consisted from a vascular layer interlocated between the axons of the ganglionic cells and vitreous humor filling the eye ball.

Discussion

The aquatic vertebrate eyes have a good vision under the water and tend to be myopic in the air, while the terrestrial vertebrate eyes have good vision in air and tend to be hyperopic under the water (Jones et al. 2007; Katzir and Howland 2003). There are many factors were related with aquatic eye to be maintain the high quality vision as; chromatic absorption of light, temperature and underwater pressure, turbidity of water.

The morphological adaptation of the teleosts visual system according to the physical and spectral characteristics of the aquatic environment makes vision under the water very different from the vision in the air. Teleosts species distributed in different depths from shallow illuminated zones to a low illuminated depth so there are many adaptations of the morphology of the eye to have good vision in the different condition under the different quality water (Collin et al. 2009).

The different morphological appearance of the eyes of the different fish-species are reflected in the shape, structures, and their location on the head as noted in many published data (Collin et al. 2009; El Bakary 2014; Schmitz and Motani 2010). Generally, it is well known that the fish species act in the dark or deep water area or at night time is characterized by the large eyes. The same results were found in the examined fish (*Sparus aurata*), have a relatively round large sized dorsolaterally located eye on the rostral part of the head that shows some mobility to increase their vision field for swimming and searching for their food, these results confirmed with that reported by (Darwish et al. 2015; Schmitz and Wainwright 2011), the large-sized eye presented to increase the sensitivity to light.

In agreement with the previous published articles, the eye of the teleost fish is consist of three layers; external, middle

and internal layer (El Bakary 2014; Genten et al. 2009). The external layer named as a sclera-corneal layer, which described as having two parts: the anterior transparent cornea and posterior opaque white sclera (Genten et al. 2009). The inner layer is named photosensitive retina, in many species of fishes is organized in ten different layers starting with the pigment epithelium layer till the inner limiting membrane (El Bakary 2014; Genten et al. 2009).

The histological examination of the cornea of *Sparus aurata* agree with that described by (Collin and Collin 1998) in the gadiform fishes, the cornea is formed from four layer; dermal Cornea, dermal stroma, mucoid layer, scleral cornea, Iridescent layer, posterior scleral stroma and anterior scleral stroma, in which cornea is divided into three layers; dermal Cornea and anterior and posterior scleral stroma. Furthermore, the mucoid layer located between the dermal stroma and the anterior part of the scleral cornea, while there is a layer named iridescent layer located between the anterior and the posterior scleral stroma as noted by (Collin and Collin 1998) in the gadiform fishes. Histological investigation of the cornea of *Sparus aurata* confirmed that the cornea is covered by an anterior stratified cuboidal epithelium layer as noted in *Siganus javus* (sadat Mansoori et al. 2014), *M. cephalus* (El Bakary 2014), and *Sardinella aurita* (Salem 2016), but there is a single cuboidal layer to a short columnar layer in aquatic frog *Xenopus laevis* (Nakayama et al. 2015).

In the current study, the pigmented epithelium layer of the retinal layer represented the non-nervous area. The current study described this layer as a thick layer as the examined fish considered one of the bottom feeder fishes as noted in *A. anguilla* (Atta 2013), *M. cephalus* (El Bakary 2014) and *S. gairdneri* (Douglas 1982), also the similar observation was confirmed by this noted by (Arnold 1977) that layer thickness is attributable to the scarcity of the light in the environment of these fish. Furthermore, (Salem 2016) in *S. aurita* reported that the pigmented layer of retina occupy nearly one third of the retinal layer thickness. The pigmented layer of photosensitive retina is exposed to incoming light; in case of dark condition by the rod cells while in case of the light condition by the cone cells. This fact is very important to enable fish to obtain their food under the light and dark condition or even when occur sudden changes in weather conditions from light to dark or vice versa (Donatti and Fanta 2002; El Bakary 2014).

The more fantastic feature is the number of photoreceptor types, their arrangement and their density within the retinal layer have an important correlation with the feeding habitat and habit and the light directions (Reckel et al. 2001). There is a relationship between the nature of the environment and the fish photoreceptor either cone cells for photopic vision or rod cells for scotopic vision. The present study described that, the *Sparus aurata* have the photoreceptor cells is consists of rods and cones which situated under the pigmented epithelium

layer as noted in many fish species as in cardinal fish (Fishelson et al. 2004). In contrast (Atta 2013) in *A. anguilla* found that this layer is consists only from the rod cells, furthermore in the same line, (El-Attar et al. 1999) noted the presence of cone cells only in *Alestes nurse* meanwhile in *O. niloticus* the photoreceptor layer is composed of cone cells and rarely rod cells as it considered as mid-water and surface dweller fish. Finally, according to (Brauer 1908), there is a condition called the dark-retina adaptation, in which the absence of the cone cells correlated with the fishes life in the water with depth more than 400 m.

In the present study, the density of rod cells in the retina is higher and effective in the visual reactions searching for food and avoiding predators as noted by (Fishelson et al. 2004) in cardinal fish, this result confirmed that the fish species with large eye having the highest number of the rod cells. In contrast, (Reckel et al. 2001) in garfish *Belone belone* reported that high density of the cone is the common feature.

The present study observed that there three types of the cone cells; single, double and triple. While, many authors recorded also three types of cones; long single, short single and double cone (Nag and Sur 1992; Salem 2016). Various species of cardinal fish (Fishelson et al. 2004) reported that there are three types of cone cells; single and two types of the double cone cells.

The most characteristic feature of the eye of the examined fish (*Sparus aurata*) was the presence of the ellipsosomes which formed from the transformation of mitochondria in the ellipsoids as reported by (Fishelson et al. 2004, 2012; Nicol 1989). The cristae are disintegrated and the mitochondria become more opaque. The transformed mitochondria act as storage sites of cytochrome c for use of the outer segments of the visual cells. The mitochondria of *Sparus aurata* cone cells are involved in the production of ellipsosomes. Transformation of numerous mitochondria into ellipsosomes might reflect the importance of ultraviolet filtration from its environment (Fishelson et al. 2004; Nicol 1989).

Compliance with ethical standards

Conflict of interest None.

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