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Comparative Ultrastructural Study of the Retina in Three Marine Teleost Fishes *(Hippocampus Hippocampus, Solea Solea and Gobius Niger)*

Samah T. Darwish[1], Mahmoud E. Mohalal[2], Menna M. Helal[1]

[1]Zoology Department, Faculty of Science, Al-Arish University, Egypt
[2] Zoology Department, Faculty of Science, Suez Canal University, Egypt

Abstract: Despite the fact that the fundamental structure of the retina is comparable over all vertebrates, high changeability in particular highlights of the fish retina mirrors the distinctions in visual microhabitat of these species. The vertebrate retina is the initial phase in the neural coordination of visual data. Three marine teleost fishes inhabiting various marine depths specifically ***Hippocampus hippocampus***, ***Gobius niger*** and ***Solea solea*** (Linnaeus, 1758) were investigated in a relative investigation of the fine structure of the retina. The ultrastructure and association of the components show in all the retinal layers have been examined. The retina demonstrates every one of the eight layers and two membranes common place of vertebrates. The current results demonstrated different structural components with characteristic photoreceptors mostly of either rods for ***Hippocampus hippocampus***, and ***Solea solea*** or duplex type, rods and cones, photoreceptors are identified in ***Gobius niger.*** It can be concluded that the retina of the studied teleost fishes exhibited obvious varying structure reflecting the ultrastructural characteristics for stabilizing functional characteristics of visualization affording to the marine habitat depths.
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1. Introduction

Visualization is the essential sense organ to satisfy the significant practices such as protection, nourishment, reproduction and predation (Kim *et al.,*2014; Al -Adhami *et al.,* 2010; Sattari *et al.,*2012 and Begum *et al.,* 2013). It is normally used to maintain school structure (Bone & Moore 2008). Absorption of light by aquatic marine environment which is decreased steadily in association with increasing in the depth of water which turned to become blue at 50 meters (Lythgoe, 1979). Marine teleost fishes living in various depths of marine environment revealed fluctuating optometric (Schartau *et al.,* 2009) and retinal structures (Frohlich & Wagner 1996 and Heb, 2009).

One of the most simply manageable parts of the central nervous system in vertebrates is the retina. The various teleostean retina characteristics attracted the attention of numerous scientists (Donatti & Fanta, 2007 and Al-Adhami *et al.,* 2003). The specific characteristics include the variable and enormous photoreceptors (Cameron & Easter 1995, Al-Adhami & Mir 1999 and Reckel & Melzer, 2003), the peculiar, mosaic arrangement of photoreceptors (Cheng & Novales Flamerique 2007 and Kim & Park 2016), the retinomotor mobility that include several constituents of the retina (Donatti & Fanta 2007, Braekevelt *et al.*, 1998 and McCormack & Donnell, 1994). Finally, the retinal regeneration capability (Cameron, 2000) and progressing development is well documented (Cameron & Carney, 2000 and Raymond & Hitchcock, 1997).

Like different vertebrates, the teleost retina is comprised of six unique kinds of neurons and one sort of glia cells. Retinal ganglion cells (RGC) are the main neurons to be produced trailed by different classes of neurons (Al-Adhami *et al.,* 2003). All vertebrate retina contains no less than two sorts of photoreceptors—the natural rods and cones. Rods are large utilized for low-light vision and cones for sunshine, brilliant hued vision. Animal eye variations reflect the diverse adaptations to their different environments. The retina of most fishes, frogs, turtles as well as birds have three to five kinds of cones and thus great color vision. Cold blooded vertebrates such as reptiles and fishes should be dynamic in the warm daytime (Donatti & Fanta 2007). The common structure of retinal of teleost fishes, has both rod cells (scotopic vision) and cone cells (photopic vision) and demonstrates a diversity of shape (Fernald, 1988 and Bowmaker, 1995) Occasionally, like basic contrasts in a few fishes are deliberated as biological and natural environments comprising nourishing propensities and photic environments (Fernald,1982; Kunz,1980 and

Lyall, 1957). what's more, the chasing action of the predatory fish (Kim & Park, 2016).

The retinal pigment epithelium (RPE) is the furthest (sclera) layer of the vertebrate retina. Normally, it comprises of a solitary layer of cuboidal to low columnar epithelial cells, and is identified by its characteristic possession of the brownish melanin pigments. The individual cells appear to taper at the apex, they possess numerous fine cytoplasmic processes that interdigitate with photoreceptor outer segments (Nag, 1994). Basally, the RPE is highly infolded, especially in mammals (Braekevelt, 1988), whereas in teleosts, this region is reported to be relatively smooth (Collin *et al.,* 1996). The choroidal circulation is responsible for supplying nutrients to the outer retina, especially to the RPE and photoreceptors, whereas the retinal circulation provides with nutrition to the inner retinal layers (Delaey & Van de Voorde, 2000).

Vision starts when photons are consumed by photoreceptors in the retina. Rods intercede scotopic vision and mostly have long, barrel - shaped external portions. Cones intervene photopic, high keenness vision, and often have shorter, more cone - shaped external segments. They can exist as single cells or into coupled gatherings as copies or even triples (Sandstrom, 1999).

In water, the accessible range of light changes with depth. wavelengths either short or long are all the high emphatically absorbed, and short wavelengths are extra firmly dispersed than the center of the range, thus the light at bottom is ranged from blue or from blue to green (475 nm) (Jerlov, 1976). UV photons remain found at depth in any case, and in non-turbid water they may be useful for visualization to a many hundred meters (Frank & Widder, 1996). Most diurnal fishes are known to have UV impermeable lenses and a few animal categories likewise have corneas which retain UV radiation (Orlov & Gamburtzeva, 1976 and Douglas *et al.*, 1989). The concentration of pigments in particular of these UV absorbing visual media is high to the point that the lenses or corneas seem yellow in color (Muntz, 1973).

In a previous published work, a histological observation of the retina of five different marine fishes including the present selected species were carried out (Darwish, *et al.,* 2015). There were marked variations of the retinal thickness among the species. The retinal pigmented epithelium and outer nuclear cells become more thickened in *Solea solea*, less dense in *Gobius niger* as well as finely distributed in *Hippocampus hippocampus*. Hyperpigmentation facilitates digestion of apical tips of the outer segment photophores which are rich in lipid materials. The presence of mixed rods and cones in the photophores of *G. niger* compared to single, double and trible rods in *H. hippocampus* and *S. solea* which facilitated adaptation of vision in their living habitats. Furthermore, our published study included the protein analysis of the retina and exhibited marked variation in expressed protein bands. Also, it revealed a variable expressions of their retinal isoenzyme pattern among the studied teleosts fishes.

Therefore, the objective of the current study is to find out more information of vision process and illustrate the ultrastructural variations in order to understand the functional anatomy of the retina and its relation with the different depths.

2. Materials and Methods

The collected samples of teleost fishes were obtained from Mediterranean Sea regions nearby Port Said in the Northeast of Egypt. The caught fishes were of relatively comparable sizes. The inspected fishes are:

1. ***Hippocampus hippocampus*** (Linnaeus, 1758), belonging to order Syngnathiformes and Family Syngnathidae. It`s habitat at 14 - 40 m depth (Kim & Park, 2016).
2. ***Gobius niger*** (Linnaeus, 1758), belonging to order Perciformes and Family Gobiidae. It`s habitat at 1 - 75 m depth (Braekevelt *et al.,* 1998).
3. ***Solea solea*** (Linnaeus, 1758), belonging to order: Pleuronectiformes and Family: Soleidae. It`s habitat at 0 -150 m depth (Raymond & Hitchcock, 1997).

They were conveyed to the research center in holders loaded with sea water and raised in aquaria loaded with a similar water untill the time of dissection. Eyes of the selected fishes were immediately enucleated. The retinas were readily dissected out by aid of a dissecting magnifying lens. For ultrastructural investigation, retina were cut into little portions, settled in 2.5% gluteraldehyde in 0.1M sodium cacodylate cradle at pH 7.3, post fixed in 1% OsO4 in a similar buffer, in acetone they dehydrated and dipped in Spurr's resin. The samples were cut into thin sections by Ultratome, The uranylacetate and lead citrate were used as a staining for these sections to be examined at JOEL 100CXI Transmission electron microscope (musashino3-chome Akishima Tokyo 196- 8558, Japan).

3. Results

Following examining the ultrastructural components of the retina in ***Hippocampus hippocampus, Gobius niger,*** and ***Solea solea.*** we found varying structural components. In the examined teleost fishes, Retinal pigment epithelium (RPE) comprises of a solitary layer of firmly pressed, polygonal, and low columnar epithelial cells. These epithelial cells characterize by extensive, vesicular nucleui, both smooth endoplasmic reticulum and mitochondria are well advanced. RPE lies between the photoreceptors and the choroid with characteristic blood capillaries forming the blood-retinal barrier. There are a contact between the basal surface of the RPE and a basement membrane called Bruch's film and the chorio capillaries, a layer of fenestrated blood capillaries. RPE cells display melanin pigments contained inside the cytoplasm in membrane - wrapped granules called melanosomes. The apical surface of the RPE forms several long appendages, which reach up between the external portions of the photoreceptors and mostly wrap them. Several phagosomes which are found in the cytoplasm of these cells engulf the detached parts of the external fragments. Phagosomes are more abundant in ***Solea solea*** and ***Gobius niger***. There are two sorts of pigment granules in these cells, particularly those parts that reach out between the external parts of the visual cells. These two types are round and ellipsoid-spherical-shaped structure. In between the phagosomes, there is a digested outer segment of photoreceptors (Fig. 2, A and Fig. 3 A-C).

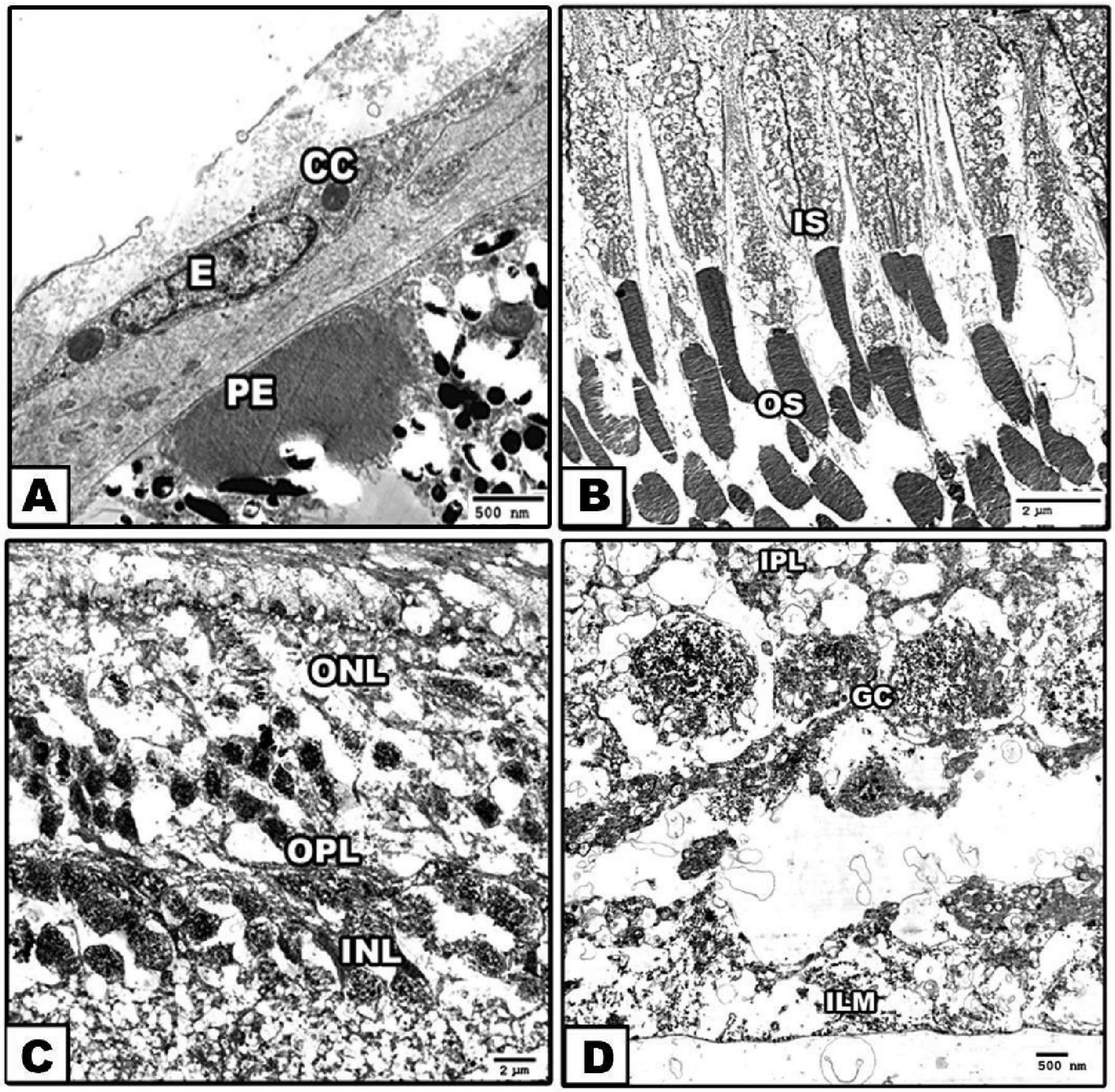

Fig. 1. Transmission electron micrographs of retina of ***Hippocampus hippocampus*** showing: A. Retinal pigmented epithelium with underlying choriocapillaries. B. Double rod with inner and outer segment. C. Outer and inner nuclear layers separated by outer plexiform layer. D. inner plexiform layer underneath the nerve fiber layer containing ganglion cells. Lead citrate and Uranyl acetate. Abbreviations: CC: choriocapillaries; E: erythrocytes; PE: pigment layer; OS: outer segment; IS: inner segment, ONL: outer nuclear layer, OPL: outer plexiform layer, INL: inner nuclear layer, IPL: inner plexiform layer, GC: ganglion cell, INL: inner limiting membrane.

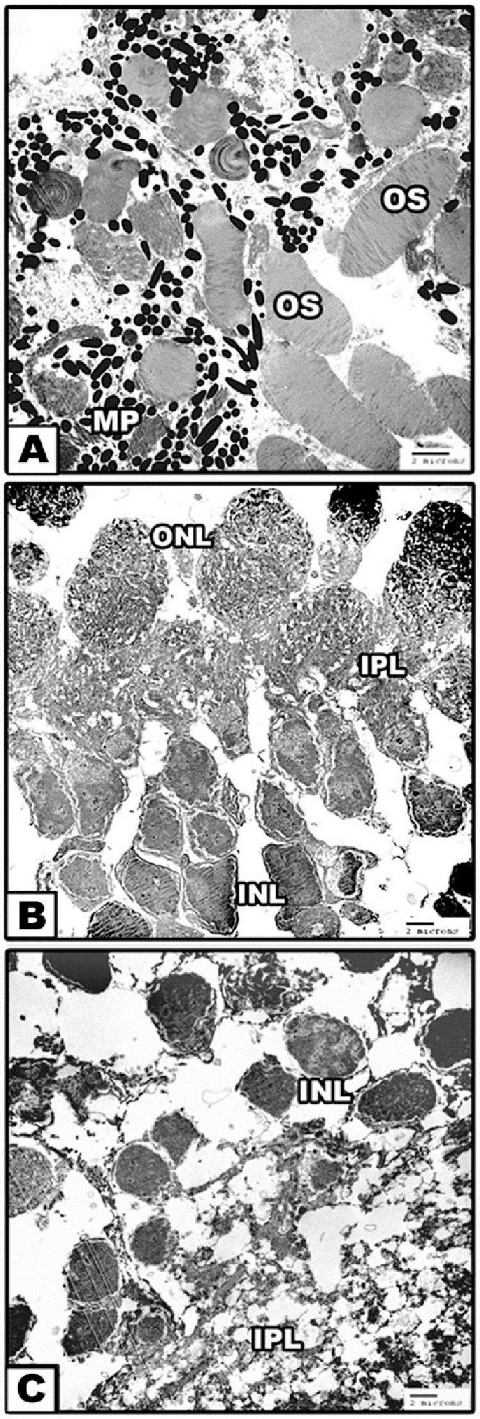

Fig. 2. Transmission electron micrographs of retina of ***Gobius niger*** showing: A. Pigmented epithelium with densely grouping oval and ellipsoid melanosomes surrounding digested outer segment photoreceptors and numerous phagosomes appear widespread in between photoreceptors. B. Outer and inner nuclear layers separated by differentiated outer plexiform layer. C. Inner nuclear layer and inner plexiform layer underneath the nerve fiber layer containing ganglion cells. Lead citrate and Uranyl acetate. Abbreviations: OS: outer segment; IS: inner segment, ONL: outer nuclear layer, INL: inner nuclear layer, IPL: inner plexiform layer.

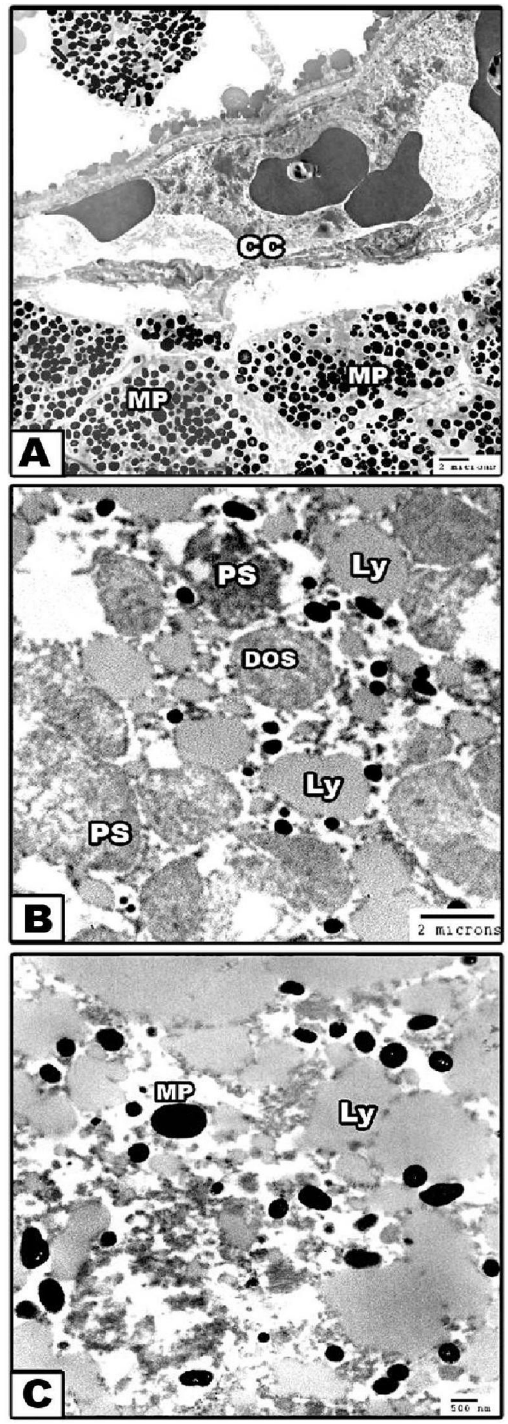

Fig.3. Transmission electron micrographs of retina of ***Solea solea*** showing: A. Pigmented epithelium with underlying choriocapillaries with abundant melanin pigments. B. Retinal pigmented epithelium with abundant lysosomes and phagosomes surrounding digested outer segment photoreceptors. C. Retinal pigmented epithelium with numerous melanin pigments, lysosomes and melanosomes. Lead citrate and Uranyl acetate. Abbreviations: CC: choriocapillaries; PS; phagosomes, MP: melanin pigments, DOS: digested outer segment and LY: lysosomes.

In ***Hippocampus hippocampus***, a characteristic retinal photoreceptors formed mainly of single and double rods conjugated at the inner segment. Outer segment is slender with pointed end and formed internally of finely arranged stacked membranes (Fig.1, B). However in ***Gobius niger*** which showed duplex type retina containing both cones and rods and in ***Solea solea*** which formed mainly of double and triple rods, but not investigated by electron microscopic investigations. Their outer segments were distributed in between RPE microvilli and formed mainly of tubular branches with lamellated inner compartment (Fig.2, A and Fig.3 A-C).

In *Gobius niger,* the outer plexus layer which lies between the outer and inner nuclear cells is thinner and less differentiated than its counterpart in ***Hippocampus hippocampus*** (Fig.1, C and Fig. 2, B, C).

Sparse distribution of outer nuclear cells was observed in ***Hippocampus hippocampus*** comparing with densely grouping outer nuclear cells (Fig.1, C).

Similar pattern of distribution of inner nuclear and ganglion cells was detected in the studied teleost specimens (Fig.1, C, D, and Fig.2, B, C).

4. Discussion

Various visual needs of teleost species may emerge from a diversity in the light conditions, feeding manners, and all levels of competitions. The attributes of lighting, light passage through the medium, and reflectance of items may change according to environments. Teleost fishes eyes develop all through its life time without affecting its visual performance. This happens due to a group of new acclimatization in the development and growth of the eye. Stretching the existing retina and the generation of new tissue at the germinal retinal zone at the margin of the eye are responsible for the expansion of retinal area (Fernald, 1990). The RPE showed characteristic digiti form processes between photoreceptor cells. It appeared bounded with dark-brown melanosomes, becoming additional concentrated in ***Solea solea*** and ***Gobius niger*** matching with ***Hippocampus hippocampus***. In ***Hippocampus hippocampus*** and ***Solea solea*** indicated that the photoreceptor layer consisted mostly of single, double and triple rods. The nuclei of the photoreceptors of the outer nuclear layer appeared more dense in ***Solea solea,*** less dense in ***Gobius niger***, as well as finely distributed in ***Hippocampus hippocampus.*** The nerve fibers and ganglion demonstrated consistent arrangement of ganglion cells, becoming high copious in ***Solea solea*** species.

Unlike ***Solea solea*** which are teleost fishes favouring the existing at deeper levels of sea water, ***Hippocampus hippocampus*** is distinguished in coastal ponds with strong oceanic effects (Darwish *et al.,* 2015 and Foster & Vincent, 2004), other forms found on soft bottoms amongst rocks and algae (Dawson, 1986). Besides, ***Hippocampus hippocampus*** is a nocturnal fish. All of these criteria supported the presence of rod photorceptors of it resemble the other deep living teleosts ***Solea solea***. Several researchers found that ***Hippocampus*** species nourish mainly during period (Hoang *et al*., 1998).

The diurnal changes in lighting conditions are what triggers photoreceptors and retinal pigment epithelium (RPE) of teleost to display major models of cell motility (known retinomotor developments). When it is dark, the pigment granules of the RPE relocate to the scleral base of the RPE cell and cone photoreceptors lengthen. In contrast, these mobilization are inverted in the light; pigment granules scatter into the long apical projections of cones contract and the RPE cell (Burnside & Basingert 1983).

The obvious rise in the thickness of RPE of ***Solea solea*** in addition to the occurrence of their densely clusters of melanosomes prevalent among lysosomes. Copiousness scattering of melanosomes assisted absorption of the apical tips of the outer segment photoreceptors which are rich in lipid materials. In some deep fishes, some investigators reported an increase in the retinal pigmented epithelium (Munk,1965) and Moray eels (Wang *et al.,* 2011). The benefits from presence of hyperpigmentation in the eye was to minimizing light scattering, enhancing absorption of stray light, and scavenging free radicals and toxic substances (Futter *et al.,* 2004). The identifying lysosomes trough RPE which may hold quite a number of about Forty hydrolytic enzymes that have been recognized by a different set of histochemical and biochemical methods (Boulton *et al.,* 1990).

The special connection between the photoreceptors and the RPE reaches out to the unusual composition of the POS layers. POS mass contains polyunsaturated lipids that are decreased by RPE phospholipases (Zimmerman *et al*., 1983) and acid lipases (Hayasaka *et al*., 1977) discharging fatty acids which subsequently reused by photoreceptors for expenditure in POS regeneration (Gordon & Bazan 1993).

The RPE induced active detaching of the tips of the light-sensitive photoreceptor, phagocytosis and external segments, in addition to its retinomotor mobility of pigment-epithelium in co-ordination with cones and rods (Kunz & Ennis,1983).

The observed findings revealed dense accumulation of melanosomes within the retinal pigmented epithelium which redistributed from the body of cell into its apical processes. The melanosomes appeared either spherical or ellipsoid structure. Elliptical melanosomes were placed equivalent to the oval or spherical melanosomes were organized obliquely or vertically to the apical processes. Melanosomes are lysosome-related organelles (Raposo & Marks 2007).

In amphibians and fish, the melanosomes of the RPE display a sensational reorganization from the cell body into the apical projections upon the beginning of light, which is opposite in the dark. On the other side, Melanosomes present in the RPE of mammals are expected not to change with the light cycle (Burnside & Laties, 1979). Melanosomes was establish to hold high combination of acid phosphatase (Nakagawa *et al.,* 1984).

Investigation of both nocturnal and diurnal forms from amongst 15 species of cardinal fish (Apogonidae), showed that the nighttime species carried bigger retina and eye associated with diurnal fishes. The daylight fishes have cones which frame a mosaic including 4 paired with one single in the middle, a model that is less noticeable at the borders of the retina and all the more so in (Fishelson *et al.,* 2004). The round shape excludes all deviated and slanted abnormalities. Retinal curviture makes it needless to splayed the pictures and neural mechanism can rectify for picture deformations without loss of data (Kroger, 2013).

The current obtained results pointed to apparent fluctuating retinal structure in the deliberate teleost species according to the marine habitat depths.

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