Comparative Studies on the Histology of Eye Retina in Some Nile Fishes with Different Dial Activities

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ABSTRACT

Aim of the work: the present work aimed to study the comparative histology of some fresh water fishes inhabiting the Nile River in Egypt. These fishes have different dial activities; *Oreochromis niloticus* is diurnal,while *Clarias gariepinus*, *Bagrus bajad* and *Chrysichthys auratus* are nocturnal. **Results:** the retinae of all studied fishes (*O. niloticus,C. gariepinus, B. bajad* and *Ch. auratus*) are composed of ten layers. These layers are the inner limiting membrane (ILM), nerve fiber layer (NFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), outer limiting membrane (OLM), photoreceptor layer (PL) and pigmented epithelial layer (PE).

The histological structure showed three types of the photoreceptors (single and double cones as well as single rods) in *Oreochromis niloticus*. The pigment epithelium layer in *C. gariepinus, B.bajad* and *Ch. auratus* completely hides the photoreceptors.

A considerable variation was recorded between the different studied fishes in the whole retinal thickness as well as their retinal layers.

Conclusion: Whole retinal thickness in *O. niloticus* (diurnal activity) is large in comparison with that of the other three siluriform fishes (nocturnal activity).

Keywords: Nile, Fish, Oreochromis niloticus, Clarias gariepinus, Bagrus bajad, Chrysichthys auratus, Eye, Retina, Histology, Diurnal, Nocturnal.

INTRODUCTION

Fish possess various kinds of sense organs and use them to detect many kinds of information in the ambient environment ^[1]. The same authors classified the sense organs in fishes into three groups: 1-organs of chemical sense which comprise the olfactory organs, taste buds and Jacobson's organ; 2- organs detect pressure change and the movement of the medium which comprise the inner ear, lateral line organ and pit organs; 3-organs of vision which include the eyes.

Vision has profound effects on the evolution of organisms by affecting survivorship through such behaviors as mating, foraging and predator avoidance^[2-6].Vision in fish depends on size and position of the eye, morphology and structure of retinal photoreceptors and structure of the pigment epithelium^[7,8].

The interspecific variations in retinal structure reflect the feeding habits and photic habitat conditions of the respective species. Rod cells provide high visual sensitivity, being used in low light conditions, while cone cells provide higher spatial and temporal resolution than rods and allow for the possibility of color vision by comparing absorbance across different types of cones which are more sensitive to different wavelengths^[9,10].

The retinal pigment epithelium is 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 ^[11].

Predaceous fish may be divided into two groups on the basis of their method of finding and procuring food; being diurnal and nocturnal predators ^[12]. In diurnal fishes, vision plays the main role in capturing prey, whereas nocturnal fishes apply the senses of smell, touch and lateral line organ. Nocturnal species show photoreceptor adaptations to generally increase their sensitivity to light, while diurnal species show adaptations to increase color discrimination ^[13,14].However, that a switch from a diurnal to a nocturnal life style is indeed possible, it has been reported for several species. ^[15-17].

The most important morphological adaptation of the visual system of mesopelagic fishes is the enlargement of their eyes compared to body size^[18]; a larger eye will increase the chance of photon capture (greater pupillary aperture), thereby allowing improved detection of body silhouettes ^[19,20].

The present study was carried out to compare between the histological structure of the eye retina of diurnal fishes (*Oreochromis niloticus*) and that of nocturnal fishes (*Clarias gariepinus*, *Bagrus bajad* and *Chrysichthys auratus*).

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MATERIAL and METHODS

1. Studied fishes:

a total of 20 specimens of four Nile fish species (5 specimens of each species) were collected from the Nile River in Egypt. These Nile fishes were:

- A.Oreochromis niloticus (Linnaeus, 1758),Family:Cichlidae,Order:Perciformes. The Nile tilapia typically feeds during davtime hours^[21].
- daytime hours^[21].
 B.Clarias gariepinus (Burchell, 1822), Family: Clariidae, Order: Siluriformes. It feeds mostly at night on active benthic organisms ^[22-24].
- **C.Bagrus bajad** (Forsskål,1775),Family: *Bagrid*ae, Order: *Siluriformes*. It feeds mostly at night and spends nearly the whole of the daylight hours in crevices of rocks^[25].
- **D.***Chrysichthys auratus* (Geoffroy Saint Hilaire,1809), Family:*Claroteidae*, Order: *Siluriformes*. It occurs usually over soft, slightly muddy substrates or substrates with heavy layers of leafy detritus in deep and relatively quiet waters ^[26].

2. Histological investigations:

the eyes of the collected specimens in the different investigated fish species were dissected and the retinae were separated from their eyes. Small pieces (5 mm) of the retinal tissue were 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 4-6 μ 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.

3. Morphometric measurements of retina:

thickness of the whole retina and its layers in the investigated fishes were measured by linear ocular micrometers. The ratio of the relationship between outer and inner nuclear layers were investigated according to the method of Wand *et al.* ^[27] and Darwish *et al.* ^[28]to determine the nocturnal or diurnal pattern of the fish.

RESULTS

1. Histological structure of eye retina:

the retinal structure of the present fishes is generally identical to that of the other vertebrates. It is composed of ten layers arranged in the following sequence (from inner to outer): inner limiting membrane "**ILM**", nerve fiber layer "NFL", ganglion cell layer "GL", inner plexiform layer "IPL", inner nuclear layer "INL", outer plexiform layer "OPL", outer nuclear layer "ONL", outer limiting membrane "OLM", photoreceptor cell layer "PL" and the pigment epithelial layer "PF"(Figures 1- 6).

The inner and outer nuclear layers appeared denser in *Oreochromis niloticus* (Figures, 1&2). The inner nuclear layer appeared less dense in *Clarias gariepinus*, *Bagrus bajad* and *Chrysithys auratus* (Figures 3-6). The ganglion cell layer was finely distributed in *Clarias gariepinus* (Figures 3&4).

The pigment epithelium covered the large part in the central region of the photoreceptor layer in *Clarias gariepinus*, *Bagrus bajad* and *Chrysithys auratus*, while in the peripheral region the pigment epithelium directed away photoreceptors. Most of the pigment granules are completely distributed and their color is brown due to the presence of melanin (**Figures 3-6**).

The cones and rods showed varying types throughout the photoreceptor layer in contact with the pigmented epithelium. In *O. niloticus*, the photoreceptors composed mainly of single and double cones (**Figure 2**). Meanwhile, single and triple cones are distinguished in *C. gariepinus* (**Figure4**).

2. Morphometric measurements:

there is a considerable variation between different studied fish species in the whole retinal thickness as well as in their retinal layers. The whole retina is more thicker (250.5±23.24) in Clarias Oreochromis niloticus; while in gariepinus, Chrysithys auratus and Bagrus bajad this retinal thicknessis clearly decreased. The inner plexiform layer in O. niloticus is very thick (55.2 ± 4.70) than that of the other siluriform fish species (C. gariepinus, C. auratus and B. bajad). Also, the thickness of the inner nuclear layer and the outer nuclear layer in O. niloticus are very large comparing with those in the other three species (Table 1).

The thickness of the photoreceptors layer in the siluriform fish species is considerably large; with an average of 108.4 ± 3.83 , 73.4 ± 2.32 and 94.6 ± 2.34 in *C. gariepinus, Ch. Auratus* and *B.bajad*, respectively. This photoreceptors layer clearly reduces (64.1 ± 16.01) in *O. niloticus*(**Table 1**).

Following assessment of the ONL/INL ration, *O.niloticus* showed the least average ratio(0.53 ± 0.07),manifesting diurnal activity. Meanwhile this average increased in *B. bajad* (2.8 ± 0.83), *Ch.auratus* (1.7 ± 0.25) and *C. gariepinus* (1.5 - 0.53) which is correlated with nocturnal activity(**Table 1**).

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		Fish species			
		O. niloticus	C. gariepinus	B. bajad	C. auratus
WR	Range	229.3-271.2	207.1 - 213.6	131.5 - 135.9	170.7 - 175.5
	Average ± SD	250.5±23.24	211.5±3.02	133.9±1.79	172.6±2.36
ILM	Range	1.1 – 1.6	1.6 – 4.3	2.7 - 3.8	2.7 – 7.1
	Average ± SD	1.3±0.28	2.7 ±1.17	3.3±0.44	4.8±2.09
NFL	Range	9.8 – 19.6	5.4 – 7.1	4.3 – 4.9	10.3 - 22.3
	Average ± SD	15.1±4.31	6.4±0.81	4.6±0.31	15.4±5.56
GL	Range	1.1 – 4.3	2.7 - 3.8	1.6 - 3.3	2.7 – 3.3
	Average ± SD	3.4±1.56	3.1±0.52	2.7±0.76	2.7±0.44
IPL	Range	50.0 - 61.4	16.8 - 22.8	12.5 - 15.8	3.8 - 12.5
	Average ± SD	55.2±4.70	19.3±2.60	14.7±1.47	7.6±4.01
INL	Range	33.7 - 39.7	3.8 – 7.1	1.6 - 3.3	4.3 – 5.4
	Average±SD	35.9±2.69	6.1±1.54	2.4±0.70	4.8±0.54
OPL	Range	31.0 - 43.5	41.3 - 45.1	11.4 – 17.3	18.0 - 24.0
	Average ± SD	37.5±6.10	43.4±1.88	14.5 ± 2.60	20.8±2.78
ONL	Range	16.8 - 21.7	6.5 - 10.2	5.4 – 7.6	7.6 - 8.6
	Average ± SD	18.9±2.23	8.2±1.48	6.4±1.11	8.1±0.54
OLM	Range	1.6 - 2.7	1.6 - 3.3	2.7 - 3.8	1.6 - 3.3
	Average ± SD	2.2±0.44	2.3±0.68	3.4±0.52	2.0±1.08
PL	Range	50.0 - 78.8	104.9 - 112.5	71.7 – 77.1	92.4 - 97.8
	Average ± SD	64.1±16.01	108.4 ± 3.83	73.4±2.32	94.6±2.34
PE	Range	10.3 - 19.6	10.9 - 16.8	8.2 - 11.4	10.9 - 13.0
	Average ± SD	14.7±4.75	13.5 ± 2.52	9.6±1.98	12.1± 0.92
ONL / INL		0.53 ± 0.07	1.5 - 0.53	2.8 ± 0.83	1.7 ± 0.25

Table 1:shows the variation	on in thickness (μ) of whole retina and its layers of the studied Nile fish specie	es.
	Fish masies	

WR: whole retina, ILM: inner limiting membrane, NFL: nerve fiber layer, GL: ganglion cell layer, IPL: inner plexiform layer, INL: inner nuclear layer, OPL: outer plexiform layer, ONL: outer nuclear layer, OLM: outer limiting membrane, PL: photoreceptor cell layer and PF: pigment epithelial layer

FIGURES





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DISCUSSION

Vision required functional integrity of retinal neural circuits of different cell types. The outer and the inner plexiform layers form synaptic relationships between photoreceptors and other bipolar and horizontal cells^[29].

Our findings revealed varying retinal thickness in the studied teleost species. Comparing with the other studied fishes, the diurnal fish, *Orechromis niloticus* showed higher retinal thickness. On the other hand, *Bagrus bajad* showed the least thickened ones. These results disagree with those of **Fishelson** *et al.*^[30] who revealed that the nocturnal forms possessed larger eye and retina compared to the other diurnal forms after investigating 15 species of cardinal fish (*Apogonidae*), including both nocturnal and diurnal forms.

The average ratio of ONL/INL increased in *Bagrus bajad*, *Chrysithys auratus* and *Clarias gariepinus* suggested the nocturnal habits which reflected the structural pattern of photoreceptors. Nile tilapia typically feeds during daytime hours; this suggests that similar to trout and salmon; it exhibits a behavioral response to light as a main factor contributing to feeding activity^[21].

According to **Munz** *et al.*^[31], *Oreochromis niloticus* showed the least average of ONL/INL. Also, our findings revealed that there is a marked increase in thickness and density of the outer nuclear cell layer in *Oreochromis niloticus* comparing with the other selected teleost fishes. These may reflect the abundant increase of photoreceptors which reflected bio-activation of the vision.

Darwish et al.^[28]studied five marine teleost fishes Hippocampus hippocampus, Sardina pilchardus ,Gobius niger ,Mullus barbatus barbatus and Solea solea. They found in S. pilchardus, the photoreceptors composed of single and double cones, H. hippocampus, M. barbatus and S. solea showed photoreceptor layer composed of single, double and triple rods. Meanwhile, mixed rods and cones are found in G. niger. In S. pilchardus and G. niger the ONL/INL ration decreased which is correlated with diurnal habits. Meanwhile, the average increased in H. hippocampus, M. barbatus and S. solea which is correlated with nocturnal habits. Comparing with H. hippocampus, S. pilchardus showed nonsignificant increase of whole retinal thickness and apparent thickened retina in S. solea>G. niger>M.barbatus.

Clarias gariepinus can tolerate waters high in turbidity and low in dissolved oxygen, and is often the last or only fish species found in remnant pools of drying rivers ^[32,33]. However, inactive foods, which it detects with its sensory

barbells before securing with its array of very fine teeth prior to gulping, are generally preferred ^[22,34].

The cone photoreceptor mosaic has been reported in a variety of species but appears to be best developed in the shallow water or epipelagic teleost retina ^[28,35,36].

The present work showed that diurnal Nile tilapia *Oreochromis niloticus* has single and double cone photoreceptors arranged in a square mosaic shape. These results agreed with that obtained by **Fishelson** *et al*.^[30]in diurnal cardinal fish (Apogonidae) ,**Lisng** *et al*.^[37] and Atta^[38]in Nile Tilapia.

The visual adaptations of *Mugil cephalus* to a thick pigmented epithelium that constitutes about one third of the retina thickness help in the protection of the rods external segments which are similar to *Salmo gairdneri*, corroborating the importance of vision for teleosts ^[36].

The pigment epithelium (PE) and photoreceptor layers of fishes were found to exhibit retinomotor movements in response to diurnal changes in lighting conditions. In darkness, the pigment granules of the PE migrate to the scleral base and cone photoreceptors elongate. In the light condition, these movements are reversed; pigment granules disperse into the long apical projections of the PE cell and cones contract^[39].

The present study showed that the pigmented epithelium was found to be very thick and distributed among the photoreceptors layer and hide it in all nocturnal siluriform fishes. This finding is in accordance with that obtained by **El-Attar** *et al.*^[40].

The retina of *Sardinella aurita* contains four basic types of photoreceptor cells; long single cones, short single cones; double cones and rods. In cross section, different cones in the retina are arranged regularly as a mosaic pattern where each pattern of the mosaic consists of four double cones surrounding a single cone. Pigmented epithelium lies between the choroid layer and the neural retina where the processes of their cells reach the outer segments of the photoreceptor cells. It is formed of a single layer of heavy pigmented columnar cells^[41].

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