Comparative Structural and Functional Study on the Eye of Freshwater Teleosts: *Clarias gariepinus, Malapterurus electricus, Anguilla anguilla* and *Oreochromis niloticus*

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Vision is a complex process in teleost fishes in exhibiting both nocturnal and diurnal behavi- freshwater teleosts were collected from the Rive include three nocturnal species, <i>Clarias garia</i> (Malapteruridae) and <i>Anguilla anguilla</i> (Angu- <i>niloticus</i> (Cichlidae). The ocular regions of the s cornea and retina were processed for histologic electron microscopy investigations. Morpholog varied between the species. The lens exhibited and socket structure densely grouped in <i>Clar</i> compared to less compacted fibers in <i>Anguilla an</i> lens angles in <i>Malapterurus electricus</i> . The cor clear in the other examined fishes. The retinal increased in the nocturnal fishes compared to the the nocturnal fishes were composed mainly of neural circuit of retinal cells showed comparat Müller cells in between the ganglion and inm <i>M. electricus</i> . These data suggest that noctur. <i>A. anguilla</i> and the diurnal fish <i>O. niloticus</i> exh and retinal structures, accommodated to their d	habiting different habitats, especially those iors. In the present study, four species of r Nile at Dakahlia Governorate, Egypt. These <i>epinus</i> (Clariidae), <i>Malapterurus electricus</i> iillidae), and one diurnal fish <i>Oreochromis</i> elected teleosts were dissected and their lens, al, morphometric, scanning and transmission ically, the gross structure of the ocular region organized lens fibers with characteristic ball <i>rias gariepinus</i> and <i>Oreochromis niloticus</i> <i>nguilla</i> , becoming widened at both peripheral nea was pigmented in <i>Anguilla anguilla</i> and thickness and visual acuity were markedly e diurnal species. Also, the photoreceptors of rods and few single and double cones. The ively increased distribution of amacrine and hal fishes <i>C. gariepinus</i> , <i>M. electricus</i> and nal fishes <i>C. gariepinus</i> , <i>M. electricus</i> and ibited differences in their corneal, lenticular tiurnal and nocturnal behaviors.		
Key words: Freshwater fishes, lens, cornea, ret	ina, photoreceptors.		
Ahmed A. EL-MANSI [™] , Biology Department, Fact Saudi Arabia. E-mail: aelmansi@kku.edu.sa Ahmed A. EL-MANSI, Yosra A. FOUDA, Dalia A. S. Mansoura University, Mansoura, Egypt. Yosra A. FOUDA, Biology Department, Faculty Arabia.	ulty of Science, King Khalid University, Abha, ABRY, Zoology Department, Faculty of Science, of Science and Arts, Baha University, Saudi		
	 Published online September 21, 2018 EL-MANSI A.A., FOUDA Y.A., SABRY D.A. 2 study on the eye of freshwater teleosts: <i>Ch Anguilla anguilla</i> and <i>Oreochromis niloticus</i>. It Vision is a complex process in teleost fishes in exhibiting both nocturnal and diurnal behav freshwater teleosts were collected from the Rive include three nocturnal species, <i>Clarias garia</i> (Malapteruridae) and <i>Anguilla anguilla</i> (Angu <i>niloticus</i> (Cichlidae). The ocular regions of the scornea and retina were processed for histologic electron microscopy investigations. Morpholog varied between the species. The lens exhibited and socket structure densely grouped in <i>Cla</i> compared to less compacted fibers in <i>Anguilla</i> and lens angles in <i>Malapterurus electricus</i>. The conclear in the other examined fishes. The retinal increased in the nocturnal fishes compared to the nocturnal fishes were composed mainly of neural circuit of retinal cells showed compared to the diurnal fish <i>O. niloticus</i> exh and retinal structures, accommodated to their of Key words: Freshwater fishes, lens, cornea, rett <i>Ahmed A. EL-MANSI</i>, <i>Fosra A. FOUDA</i>, <i>Dalia A. S. Mansoura University, Mansoura, Egypt. Yosra A. FOUDA, Biology Department, Faculty Arabia.</i> 		

Vision and ocular structure of teleosts has a wide range of adaptation for high sensitivity and visual resolution in specific environmental conditions of diurnal and nocturnal behaviors.

The functional and structural evolution of the eye is considered to be driven by the ambient light levels. Diel activity pattern controls the visual environment of teleosts, with day-active (diurnal) fish active in well-illuminated conditions, whereas night-active (nocturnal) fish cope with dim light (SCHMITZ & WAINWRIGHT 2011). Also, it has profound effects on survival via regulation of mating, foraging and predator avoidance (BOWMAKER 1991; YOKOYAMA & YOKOYAMA 1996).

The eel (*Anguilla anguilla*) is a nocturnal fish, inactive during the day and occurring under rocks, or in aquatic vegetation or sediments. *Malapterurus electricus* is also a nocturnal catfish that inhabits the River Nile and lives in shallow water, with muddy or sandy bottom neighboring rocky areas

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(VAN GINNEKEN & MAES 2005; AZEROUAL *et al.* 2010). *Clarias gariepinus* is nocturnal and widespread throughout the River Nile (ANOOP *et al.* 2009). On the other hand, the Nile tilapia *Oreochromis niloticus* is diurnal, however some are nocturnal, and a few display an arrhythmic pattern (VERA *et al.* 2009).

The cornea of teleosts is the anterior transparent window in the sclera and is highly specialized compared to other vertebrates. The cornea is characterized by its changeable coloration due to the presence of specialized chromatophores, localized mainly at the border between the cornea and sclera (GNYUBKINA & LEVIN 1987; ORLOV & KONDRASHEV 1998). During adaptive vision, the corneal pigments are shifted into the cell processes, which extend into the centre of the cornea just covering the pupil zone (GAMBURTSEVA *et al.* 1980).

Interspecific variation in the retina of teleosts reflects the feeding habits and photic habitat conditions of the respective species. Rod cells provide high visual sensitivity, being used in low light conditions; meanwhile cone cells provide higher spatial and temporal resolution than rods (AL-ADHAMI *et al.* 2010).

The retinal pigment epithelium is optimally exposed to incoming light through rods and cones. This capacity might be important to enable the fish to find their prey under light and dark conditions. A high density of cones suggests relatively good photopic visual activity and may indicate diurnal activity (DONATTI & FANTA 2002).

The retinal structure varies between teleosts according to their retinomotor movements via migration of cones, rods and pigment epithelium in relation to the light conditions (BURNSIDE 2001). The aquatic environment possesses a wide diversity of photic conditions differing in aspects such as color, clarity and turbidity (BOWMAKER 1995). A wide variety of visual adaptations found in teleosts, particularly in eye structure and retinal cellular configuration, are often related to their life habits and photic environment of the species (RECKEL & MELZER 2003).

Teleost eyes are adapted to dark conditions and light exposure via alterations of the distribution of melanin granules (BURNSIDE 2001) and shape of both lipid droplets and melanosomes (BRAEKEVELT *et al.* 1998).

The present study highlights the structural and functional adaptation of different ocular structures of freshwater teleosts living in nocturnal habitats such as *Clarias gariepinus*, *Malapterurus electricus* and *Anguilla anguilla* compared to the diurnal *Oreochromis niloticus*.

Materials and Methods

Ten freshwater samples of *Clarias gariepinus*, *Malapterurus electricus*, *Anguilla anguilla* and *Oreochromis niloticus* were collected from the River Nile (Damietta branch) at Dakahlia Governorate, Egypt during spring and summer seasons of 2015-2016 and transported to our lab. The animal handling was in accordance to the guidelines of the Institutional Animal Care and Use Committee (IACUC), Faculty of Science- Menoufia University, Egypt, Approval No: MNSA161.

Fish samples were photographed and their body weights and total body lengths were measured. They were anesthetized with MS-222 (50 ppm), and then eyes were enucleated and their horizontal and vertical diameters were measured with a microcalibrater to a precision of 0.2 mm as shown in Table 1. Later, the eye cups were dissected and their cornea, lens and retina were isolated, immediately fixed and processed for the following investigations:

Histological investigation

Cornea and retina of the selected fishes were fixed in 10% phosphate buffered formalin (pH 7.4). They were dehydrated in ascending grades of ethyl alcohol, cleared in xylene and mounted in molten paraplast at 58-62°C. Five μ m histological sections were carried out, stained with hematoxylin and eosin and investigated under bright field Olympus light microscope.

Morphometric assessments

Whole retinal thickness and retina layers were measured for different retinal regions around the central and mid-peripheral areas using a linear ocular micrometer (0.01 mm). The average ratio between outer and inner nuclear layer (ONL/INL) was recorded which could be used as an indicator of nocturnal or diurnal species according to WANG *et al.* (2011).

Scanning electron microscopy

Cornea and lens were fixed in 2.5% glutaraldehyde in cacodylate buffer (pH 7.4), dehydrated in ascending percentages of ethyle alcohol, dried in critical point Polaron apparatus and coated with gold in Ion Sputter model 3 Pelco 91000. The samples were examined and photographed at different angles using a Jeol scanning electron microscope at accelerating voltages of 5 or 10 kV.

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Species	Weight (g)	Length (mm)	Eye diam	Eve size	
			HzD	VerD	29 • • • •
Cg	310±20	290±20	$4.8{\pm}0.08$	$4.8{\pm}0.08$	18.09
Ме	330±25	335±25	4.2±0.05	4.2±0.05	13.85
Aa	410±50	750±45	5.2±0.09	5.2±0.09	21.23
On	180±20	210±15	9.4±1.02	10.2±1.12	75.30

Mean weight (g), length (mm), eye diameter and size of *Clarias gariepinus*, *Malapterurus electricus*, *Anguilla anguilla* and *Oreochromis niloticus*

Each result represents the mean±SE (n=10).

Abbreviations: Aa - Anguilla anguilla; Cg - Clarias gariepinus; HzD - Horizontal diameter; Me - Malapterurus electricus; On - Oreochromis niloticus; VerD - Vertical diameter. Size of eye calculated according to (πr^2) .

Transmission electron microscopy

Fresh retinal samples were fixed in 2.5% glutaraldehyde in cacodylate buffer (pH 7.4, post-fixed in 1% osmium tetroxide, dehydrated in ascending percentages of ethyl alcohol, cleared in propylene oxide and embedded in resin. Semithin sections (1 μ m) were cut using an Ultracut Reichert-Jung ultramicrotome, stained with toluidine blue and examined under a light microscope. For electron microscopy, ultrathin sections stained with uranyl acetate and lead citrate were examined with a Joel CX 100 transmission electron microscope operated at an accelerating voltage of 60 kV.

Statistical analysis

Data were presented as mean \pm standard error (SE) and analyzed using one-way ANOVA followed by the Tukey-Kramer test performed using SPSS (version 18). For single and double cones, differences between groups were calculated using the paired sample *t*-test, considered significant at P<0.05.

Cell counts were converted to density counts in cells (100 μ m). Retinal areas were defined from scanned images of the retinal outlines and the software program Image J (Rasband, 1997-2005). Total cell numbers were calculated by multiplying the median cell density of each contour by the retinal area of that contour. Photographs of the retina were captured at every alternate sampling site using an Olympus digital camera and DP70-BSW software (Olympus Corp., Japan).

Results

Morphological criteria of ocular region

In *Oreochromis niloticus*, the pupil is darkened and ensheathed by a circular ring of the iridis. The ocular size was larger in *O. niloticus* and bulged at its dorsal margin compared to the other studie d species which were relatively smaller in size (Table 1, Fig. 1 A-D1).

Cornea histological structure and scanning electron microscopy

In the studied species, the outer corneal layer was composed of non-keratinized stratified epithelium. Its basal columnar cells rest on a basement membrane. A thin sheath of Bowman's layer was detected underneath the epithelium. Ovoid lamellate bodies are distributed within the epithelium of M. electricus. Also, a dense dark-brown melanin aggregation was detected within the epithelium of A. anguilla. The corneal stroma occupied a large space and was composed of regularly oriented collagen fibrils revealing its softness and flexibility. The collagen fibrils were fenestrated by keratocytes. Numerous elliptical melanosomes were distributed throughout the peripheral stromal region of A. anguilla. At the end of the stroma, Descemet's membrane with its underlying endothelium was noted (Fig. 2 A-D1).

Scanning electron microscopy of lens

The lens of the investigated freshwater teleosts has a circular structure. The lens is coated by a thin cellular sheet of collagen fibers. In *C. gariepinus* and *O. niloticus*, the lens fibers were arranged in concentric layers of densely packed lens fibers representing the superficial and deep cortical fibers. It is composed mainly of tightly joined parallel ribbon-like structures with minimal intercellular spaces. The lens fibers interconnected with each other by well developed ball and socket structures. However in *M. electricus*, the lens fibers were grouped in the middle region and were widely separated at both ends. In *A. anguilla*, we observed



Fig. 1. Photomacrographs showing lateral views of selected teleosts. *Clarias gariepinus* (A,A1), *Malapterurus electricus* (B,B1), *Anguilla anguilla* (C,C1), *Oreochromis niloticus* (D,D1); scale bar: A-D, 6 cm; A1-D1, 3 cm.

loose interlocking between balls and sockets of the lenticular fibers. Also, the lens fibers develop globular elements which appear as elevations of the general membrane surface. In all the specimens, the lens fibers were interconnected into planar sheets with organized ball and socket structures on short edges allowing lens movement (Fig. 3 A-D1).



Fig. 2. Photomicrograph showing histological sections (A-D) and scanning electron micrographs (A1-D1) of cornea of *Clarias gariepinus* (A,A1), *Malapterurus electricus* (B,B1), *Anguilla anguilla* (C,C1), *Oreochromis niloticus* (D,D1). Note dense distribution of melanosomes within epithelium as well as ovoid bodies (arrow head) scattered in between the peripheral stromal region in *Anguilla anguilla*. Abbreviations: Ep – epithelium; BM – Bowman's membrane; DM – Descemet's membrane; me – melanosomes; St – stroma.



Fig. 3. Scanning electron micrographs showing lens of *Clarias gariepinus* (A&A1), *Malapterurus electricus* (B,B1), *Anguilla anguilla* (C,C1), *Oreochromis niloticus* (D,D1). Abbreviations: B – ball; C – cornea; L – lens; LF – lens fibers; P – pit; R – Retina; S – socket.

Morphometric observations

Retinae of the studied freshwater fishes are composed of nine layers including a pigment epithelial layer (PE), photoreceptor layer (PL), outer limiting membrane (OLM), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL) and nerve fiber layer (NFL). The retinal thickness varied markedly between the studied species. Compared with the diurnal O. niloticus, the nocturnal fishes C. gariepinus, M. electricus and A. anguilla showed a significant increase of whole retinal thickness, being more pronounced in C. gariepinus. Moreover, the thickness of the photoreceptor cell layer was higher in the nocturnal fishes and attained a maximum increase in C. gariepinus. The outer and inner nuclear layers were markedly thickened in A. anguilla compared to the other species.

The mean density of rods, single and double cones (cells per 100 μ m) was significantly different across the four different species. *O. niloticus* had the smallest number of rods and highest numbers of single and double cones while the cell densities of ganglion cells were significantly higher in *C. gariepinus* and *A. anguilla* compared to *O. niloticus*. Following assessments of the ONL/INL ratio, it was found that *O. niloticus* exhibits the lowest average value, manifesting its diurnal activity in comparison to the other studied teleosts (Table 2, Figs 4-6 & 7 A-D).

At the light microscopic level, haematoxylin, eosin and toluidine semithin histological sections revealed that the retinae of the studied freshwater fishes are composed of nine layers as previously explained. The retinal pigment epithelium is composed of a single layer of cuboidal cells attached basally and laterally with the photoreceptor outer segments, which contain mainly rods in nocturnal fishes such as C. gariepinus, M. electricus and A. anguilla. However, strikingly few single and double cones were present in A. anguilla. Melanosomes are densely grouped in the pigment epithelium and photoreceptors of C. gariepinus and M. electricus, compared to A. anguilla and less dense in O. niloticus. The inner nuclear cells exhibited clearly differentiated bipolar, horizontal and amacrine cells in O. niloticus compared with the other species. The outer nuclear cells are also more dense in O. niloticus (Figs 7 A-D, 8 A-A3, 9 A-A4, 10 A-A3, 11 A-A4).

At the ultrastructural level, the pigment epithelium is composed of a single layer of columnar cells interdigitated with the photoreceptor outer segments which are characterized by regularly oriented stacked membranes. The cytoplasm of pigmented epithelium contains abundant melanosomes and cytoplasmic organelles. A dense distribution of myoid bodies occurred in between the photoreceptors in *C. gariepinus* and *M. electricus* compared to *A. anguilla*. The lowest number of myoid bodies was found in *O. niloticus*. Bruch's membrane is relatively thin and homogeneous. The choriocapillaris possessed a fenestrated endothelium. In *O. niloticus*, the pigment epithelium

Table 2

	Retinal cell layers thickness (µm)								
	R	PL	ONL	OPL	INL	IPL	GCL	NFL	ONL/INL Ratio
Cg	220.8±8.32 ^a	104.2±4.32 ^a	34.6±2.12 ^a	19.1±1.61 ^a	$20.8{\pm}1.17^{a}$	$29.4{\pm}1.74^{a}$	$4.2{\pm}0.42^{a}$	$8.5{\pm}0.28^{a}$	1.67 ^a
Ме	208.7±10.21 ^{a,b}	87.1±5.11 ^b	37.1±2.45 ^a	$14.5{\pm}1.82^{b}$	$20.1{\pm}2.97^{a}$	26.7±2.81 ^a	$3.2{\pm}0.37^{b}$	$9.1{\pm}0.30^{a,b}$	1.84 ^b
Aa	206.9±7.89 ^{a,b}	62.7±3.14 ^{b,c}	47.6±3.06 ^b	18.2±1.77 ^{a,c}	$28.4{\pm}1.88^{b}$	$34.8{\pm}1.39^{b}$	5.2±0.48 ^{b,c}	$9.9{\pm}0.19^{b}$	1.68 ^a
On	185.5±7.01 ^c	44.8±3.78 ^c	41.2±2.29 ^{b,c}	13.5±1.25 ^b	26.1±1.50 ^{b,c}	43.7±1.64 ^c	5.7±0.36 ^c	$10.3{\pm}0.37^{b,c}$	1.58 ^{a,c}
F-test	2.660	7.520	2.315	6.820	13.247	4.521	3.801	2.016	2.660

Mean thickness of retinal cell layers (in µm) of *Clarias gariepinus*, *Malapterurus electricus*, *Anguilla anguilla* and *Oreochromis niloticus*

Each result represents the mean \pm SE (n=10). ^{a-c} Means in a column without a common superscript letter are significantly different (P<0.05) as analyzed by one-way ANOVA.

Abbreviations: Aa - Anguilla anguilla; Cg - Clarias gariepinus; Me - Malapterurus electricus; On - Oreochromis niloticus; GCL - ganglion cell layer; INL - inner nuclear cell layer; IPL - inner plexiform layer; NFL - nerve fiber layer; ONL - outer nuclear cell layer; OPL - outer plexiform layer; PL - photoreceptor layer; R - retina.



Fig. 4. Mean numbers of ganglion cells (GC) (cells per 100 μ m) of *Clarias gariepinus* (Cg), *Malapterurus electricus* (Me), *Anguilla anguilla* (Aa) and *Oreochromis niloticus* (On). Each result represents the mean \pm SE (n=10); * Significant at P<0.05 and ** at P<0.01.



Fig. 5. Mean numbers of rods (cells per 100 μ m) of *Clarias gariepinus* (Cg), *Malapterurus electricus* (Me), *Anguilla anguilla* (Aa) and *Oreochromis niloticus* (On). Each result represents the mean \pm SE (n=10); * Significant at P<0.05 and ** at P<0.01.



Fig. 6. Mean numbers of single and double cones (cells per 100 μ m) of *Clarias gariepinus* (Cg), *Malapterurus electricus* (Me), *Anguilla anguilla* (Aa) and *Oreochromis niloticus* (On). Abbreviations: SC – Single cone; DC – Double cone. Each result represents the mean ± SE (n=10); * Significant at P<0.05 and ** at P<0.01.



Fig. 7. Photomicrographs showing histological sections of retina of A. *Clarias gariepinus*. B. *Malapterurus electricus*. C. *Anguilla anguilla*. D. *Oreochromis niloticus*. Note increased distribution of melanosomes in pigment epithelium and in between photoreceptors of *Clarias gariepinus* and *Malapterurus electricus*. Abbreviations: BM – Bruch's membrane; Chc – choriocapillaris; GCL – ganglion cell layer; ILM – inner limiting membrane; INL – inner nuclear layer; IPL – inner plexiform layer; me – melanosomes; NFL – nerve fiber layer; OLM – outer limiting membrane; ONL – outer nuclear layer; OPL – outer plexiform layer; PL – photoreceptor layer; PE – pigmented epithelium.



Fig. 8. A-A3. Toluidine blue semithin section showing regular arrangement of retinal layers of *Clarias gariepinus*. A1. Ganglion cells. A2. Inner nuclear cells. A3. Photoreceptors infiltrated by melanosomes and in contact with pigment epithelium.B-G. Transmission electron micrographs of retina of *Clarias gariepinus*. B&C. Pigment epithelium containing melanosomes and mitochondria and basally choriocapillaris. The upper vesicles interdigitate with photoreceptor outer segment. D. Outer nuclear cells, E. Photoreceptor inner and outer segment infiltrated by melanosomes. F. Inner nuclear cells enclosing in between Müller cells. G. Ganglion cells enclosed in between Müller cells. Abbreviations: AC – amacrine cell; BC – bipolar cell; BM – Bruch's membrane; C – cone; ChC – choriocapillaris; GC – ganglion cells; ILM – inner limiting membrane; INC – inner nuclear cell; INL – inner nuclear layer; IPL – inner plexiform layer; IS – inner segment; M – mitochondria; MC – Müller cell; me – melanosomes; NA – nerve axon; ONL – outer nuclear layer; OPL – outer plexiform layer; OS – outer segment; P – phagosome; Ph – photoreceptors; PE – pigmented epithelium; R – rod.



Fig. 9. A-A4. Toluidine blue semithin section showing regular arrangement of retinal layers of *Malapterurus electricus*. A1. Ganglion cells. A2. Inner nuclear cells. A3. Outer nuclear cells. A4. Photoreceptors infiltrated by melanosomes and attached to pigment epithelium. B-G. Transmission electron micrographs of retina of *Malapterurus electricus*. B. Outer nuclear cells attached to photoreceptor inner segment containing mitochondria. C. Pigment epithelium with abundant melanosomes and mitochondria. D. Inner nuclear cells enclosed in between Müller cells. E. Photoreceptor inner and outer segment F. Melanosomes dispersed in between photoreceptor. G. Ganglion cells. Abbreviations: AC – amacrine cell; BC – bipolar cell; BM – Bruch's membrane; ChC – choriocapillaris; GC – ganglion cells; ILM – inner limiting membrane; INL – inner nuclear layer; IPL – inner plexiform layer; IS – inner segment; M – mitochondria; MC – Müller cell; me – melanosomes; NA – nerve axon; OLM – outer limiting membrane; ONL – outer nuclear layer; ONC – outer nuclear cell; OPL – outer plexiform layer; OS – outer segment; Ph – photoreceptos; PE – pigmented epithelium; R – rod; ROS – rod outer segment.

ROS - rod outer segment.



Fig. 10. A-A3. Toluidine blue semithin section showing regular arrangement of retinal layers of *Anguilla anguilla*. A1. Ganglion cells. A2. Inner nuclear cells. A3. Outer nuclear cell attached to photoreceptors containing single and double cones. B-G. Transmission electron micrographs of retina of *Anguilla anguilla*. B&C. Pigment epithelium with abundant melanosomes and mitochondria and interdigitating dorsally with photoreceptor outer segment. D. Photoreceptor outer and inner segment. E&F. Outer nuclear cells attached to photoreceptor inner segment. G. Ganglion cells. Abbreviations: AC – amacrine cell; BC – biplar cell; C – cilium; CIS – cone inner segment, DC – double cone; GC – ganglion cells; HC – horizontal cell; ILM – inner limiting membrane; INL – inner nuclear layer; IPL – inner plexiform layer; M – mitochondria; MC – Müller cell; me – melanosomes; NA – nerve axons; ONL – outer nuclear layer; ONC – outer nuclear cell; OPL – outer plexiform layer; Ph – photoreceptors; PE – pigmented epithelium; R – rod; RIS – rod inner segment; ROS – rod outer segment; SC – single cone.



Fig. 11. A-A4. Toluidine blue semithin section showing regular arrangement of retinal layers of *Oreochromis niloticus*. A1. Ganglion cells. A2. Inner nuclear cells. A3. Outer nuclear cells. A4. Photoreceptors infiltrated by melanosomes. B-F. Transmission electron micrographs of retina of *Oreochromis niloticus*. B. Photoreceptor (rods and cones) inner and outer segment infiltrated by melanosomes. C. Pigment epithelium with abundant melanosomes, mitochondria and phagosomes, interdigitating dorsally with photoreceptor outer segment. D. Inner nuclear cells. E. Outer nuclear cells. F. Ganglion cells and nerve axons.

Abreviations: AC – amacrine cell; BC – bipolar cell; BM – Bruch's membrane; BV– blood vessel; ChC – choriocapillaris; COS – cone outer segment; DC – double cone; GC – ganglion cells; HC – horizontal cell; ILM – inner limiting membrane; INC – inner nuclear cell; INL – inner nuclear layer; IPL – inner plexiform layer; IS – inner segment; MC – Müller cell; me – melanosomes; NA – nerve axons; ONL – outer nuclear layer; ONC – outer nuclear cell; OPL – outer plexiform layer; OS – outer segment; P – phagosome; Ph – photoreceptors; PE – pigmented epithelium; R – rod; ROS – rod outer segment; SC – single cone.

possessed numerous phagosomes, lysosome-like bodies, lipid droplets, and myeloid bodies. *M. electricus* exhibited abundant amacrine and Müller cells in between the ganglion and inner nuclear cells. Also, abundant mitochondria are observed in the inner segment of *M. electricus* (Figs 8-10 B-G, 11 B-F).

Discussion

The corneal epithelium is thickened in *C. gariepinus* compared to the other species. Also, a striking presence of spherical lamellated bodies in the epithelium of *M. electricus* and *A. anguilla* has a specific distribution of dense pigmentation in the epithelium and dispersed ellipsoid bodies in the upper stromal layers. The thickened corneal epithelium in *C. gariepinus* may protect against infection, meanwhile the presence of spheroid bodies in *M. electricus* serve for concise corneal curvature for light collection.

The presence of corneal chromatophores was reported in the tropical marine puffer, *Canthigaster cinctus*, and boreal whitespotted greenling, *Hexa-grammos stelleri* (KONDRASHEV & GNYUBKIN 1999) and wrasses (Labridae) (SIEBECK & MARSHALL 2000). Also, toadfishes *Tetractenos hamiltoni* and *Torquigener pleurogramma* (Tetraodontidae) have yellow corneas with characteristic pigment migration in a dorsal or ventral direction during light-and dark-adaptation (SIEBECK *et al.* 2003).

In the teleosts studied herein, the lens is usually rigid and protrudes underneath the cornea allowing the transmission of light and capturing much information to be sent to the retina. The nocturnal fishes *M. electricus C. gariepinus* and *A. anguilla* revealed an elevated ratio of ONL/INL, coinciding with increased retinal thickness in association with more organized corneal layers and grouping lens fibers compared to *O. niloticus*.

GAGNON *et al.* (2013) observed that the hatchet fishes *Argyropelecus aculeatus* and *Sternoptyx diaphana* and the barrel-eye *Opisthoproctus soleatus* possessed modified lenses which break the counter illumination camouflage of their prey.

The eyes of the nocturnal fishes are adapted to dim-light vision. Light filtration by lens pigments occurs mainly in diurnal or shallow-swimming species. All species have a similar structural arrangement of lenticular fibers. The lens is composed of a mono-layer of epithelial cells anteriorly and concentric layers of differentiated fiber cells from the periphery to the center. A similar pattern of fiber cell ribbon shapes and interdigitating knobs was reported by ZIGMAN (1990). In shallow and moderate water depths, there is sufficient light for fishes to recognize their food items. In dark furrows or caves, there is no down welling daylight so that fishes have evolved various visual system characteristics allowing them to operate under different types of photic conditions presented by increasing the whole retinal cell layer thickness and accommodation of both lenticular and corneal architecture.

The examined freshwater fishes exhibited similar characteristic features of retinal structure. There was an increase in the pigmentation of pigmented epithelium and photoreceptors of nocturnal fishes such as *C. gariepinus* and *M. electricus* that was comparatively lesser in *A. anguilla* and *O. niloticus*. The increased number of ganglion cells in nocturnal fishes compared to *O. niloticus* is the principal element of optic nerve supporting their visual processing. Although the photoreceptors of the nocturnal fishes are composed mainly of rods, few numbers of single and double cones were detected in *A. anguilla*. Similar findings were reported in *A. anguilla* (BRAEKEVELT 1985, 1988 a, b) and *A. marmorata* (WANG *et al.* 2014).

LANDSBERGER *et al.* (2008) reported similar findings in African electric fish *Gnathonemus petersii*. The authors observed that *G. petersii* have hundreds of rods and lesser numbers of cones grouped together in bundles ensheathed by a tapetum lucidum. During daytime, the structure of these "macro-receptors" altered dramatically depending on the retinomotor movements. The rods and cones are located in different compartments of the bundle and separated by a narrow canal.

Our findings revealed that diurnal *Oreochromis niloticus* has single and double cone photoreceptors. These results agree with LISNEY & HAWRYSHY (2010) who reported that *Oreochromis niloticus* has single and double (twin) cone photoreceptors, forming a square mosaic in high densities.

At the ultrastructural level, the studied fishes, especially *M. electricus*, have pigment epithelium interdigitated with the photoreceptor outer segments with regularly oriented stacked membranes. The inner segment of their photoreceptors possessed dense aggregated mitochondria. A dense distribution of melanosomes and myoid bodies was noted in between the photoreceptors in *C. gariepinus* and *M. electricus* compared to *A. anguilla* and the fewest were found in *O. niloticus*. In *O. niloticus*, phagosomal activity is clearly detected within the cytoplasm of the pigment epithelium which may be of high importance for scavenging the free radicals released as a result of increased light exposure.

Hyperpigmentation of pigmented epithelium and photoreceptors is an important biological adaptation for absorbing light and minimizing its scattering, especially in nocturnal fishes; it protects against light sources and free radicals in diurnal species (FUTTER *et al.* 2004). It is known that melanosomes act as lysosome-like organelles (RAPOSO & MARKS 2007).

The neural circuit of retinal cells showed comparatively increased distribution of amacrine and Müller cells in between the ganglion and inner nuclear cells in *C. gariepinus* and *M. electricus*. Abundant mitochondria were detected in the inner segment of *M. electricus*. The increased distribution of these cells reflects the increased immune reaction of these teleost fishes (DE JUAN *et al.* 2010).

GOLDMAN (2014) reported the importance of Müller cells in maintaining retinal homeostasis and proliferation and generation of retinal neurons of teleost fish such as zebrafish; these features suggest that teleost fish possess a unique retinal environment that supports progenitor cell formation and maintenance. Müller cells have a specific role in supporting and providing structural stabilization to the retina (TURNER & CEPKO 1987).

We conclude that the studied nocturnal fishes *C. gariepinus*, *M. electricus* and *A. anguilla* and the diurnal fish *O. niloticus* exhibited differences in cornea, lens and retinal structures accommodated to their mode of living.

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Author Contributions

Research concept and design: A.A.E.M.; Collection and/or assembly of data: A.A.E.M., Y.F.; Data analysis and interpretation: D.S.; Writing the article: A.A.E.M.; Critical revision of the article: Y.F., D.S.; Final approval of article: A.A.E.M.

Conflict of Interest

The authors declare no conflict of interest.

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