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Morphological and Histological Adaptations for Retinal Structure in Eyes of the Amphibious Fish, *Alticus kirkii* and the Non-Amphibious Fish, *Gambusia affinis*

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ABSTRACT

The current research aimed to investigate the adaptations in eye morphology and the structure of retinal photoreceptors in amphibious fish (*Alticus kirkii*) and non-amphibious fish (*Gambusia affinis*) to understand the visual adaptations of *A. kirkii* upon being out of water. The morphological and histological features of the eyes of both species were adressed. Results showed that *A. kirkii* exhibits several visual adaptations for seeing in air. Morphologically, the eyes of *A. kirkii* are bulging and positioned on retractable stalks at the top of the head, unlike those of *G. affinis*. The ratio of axial length to head length is greater in *A. kirkii* than in *G. affinis*. Histologically, the cornea of *A. kirkii* is more curved than that of *G. affinis*, with a crooked internal layer that is more curled in *A. kirkii* and reduced in *G. affinis*. The lens is larger, slightly flattened, and more stable in *A. kirkii* compared to *G. affinis*. The retina is thicker in *A. kirkii* and consists of 10 layers. In *A. kirkii*, the external layer of pigmented epithelium is densely packed in the peripheral region of the photoreceptor layer, whereas in *G. affinis*, it is composed of epithelial cells in the peripheral region but is less thick. The visual cell layer includes simple and double cones; *A. kirkii* has a greater abundance and larger size of cones compared to *G. affinis*. The outer nuclear layer, which contains the visual cell bodies, is thicker in *A. kirkii* than in *G. affinis*. The outer plexiform layer is narrower in *A. kirkii*, with higher densities of both bipolar and horizontal cells compared to *G. affinis*. The inner nuclear layer shows a heterogeneous cell composition and is more crowded in *A. kirkii* than in *G. affinis*. Additionally, disparities in the thickness of the inner limiting membrane and nerve fiber layer affect the transmission of signals from the retina to the brain.

INTRODUCTION

Amphibious fishes (amph-fishes) are found throughout a variety of freshwater and marine environments in tropical, subtropical, and temperate climates **(Sayer & Davenport, 1991)**. The term "amph-fish" refers to species that are known to inhabit both aquatic and terrestrial environments and to regularly spend time outside of the water **(Gordon, 1998)**. **Gordon *et al.* (1985)** concluded that over 80% of the life of *Alticus kirki* is spent on rock surfaces above the sea's edge. Amph-fishes' terrestrial activities are intimately linked to their needs for food, mate selection, and territory defense **(Murdy, 1989)**.

Amphibian blenny, *A. kirki* is widely distributed in rocky intertidal areas around the Red Sea **(Klausewitz, 1964)**. As adults, the fish grow to a maximum length of 12cm S.L.

(standard length). They are gregarious, nocturnal, and spend most of their active lives outside of the water, capable of jumping from one pool to another, and situated close to the tidally varying water line. They engage in complicated interactions with their conspecifics and other species in their habitat, while actively grazing on algae located on rock surfaces **(Randall, 1983; Brillet, 1986; Brown *et al.*, 1991, 1992; Alabssawy & Khalaf-Allah, 2017)**. However, the question remains how amph-fish, *A. kirkii* visualize out of water.

In teleost fish, light and vision are the primary environmental factors that impact numerous essential activities of fish, including swimming, migration, food search and reproduction **(Schmitz & Wainwright, 2011; De Busserolles *et al.*, 2013; El-Bakary & Abumandour, 2017)**.

The eye, being a vital sensory organ, performs an exceptionally important function in facilitating communication between organisms and their environment (**Kassab *et al*., 2001; Oliver *et al*., 2004; Aljalaud & Azab, 2021)**. Numerous vertebrate eye characteristics are adaptations to the visual environments in which they evolved **(Land & Nilsson, 2002)**.

Fish are equipped with a variety of sensory organs that enable them to perceive and interpret a wide range of environmental stimuli. The eye's size and location, the shape and arrangement of the pigment epithelium, and the arrangement of the photoreceptors in the retina all affect a fish's vision **(Levine & MacNichol, 1982; Fishelson *et al*., 2004; Azab *et al.* 2017)**.

The variations in retinal structure between species indicate the photosynthetic environmental conditions and eating patterns of each species. In low light conditions, rod cells are used to achieve high optical sensitivity. In contrast, cone cells provide superior spatial and temporal resolution compared to rod cells and enable color vision by comparing absorption across different types of cones that are more sensitive to specific wavelengths **(Fishelson *et al*., 2004; Kondrashev, 2022)**.

There are no available studies on the morphology of eyes and structure of the retina photoreceptors to detect how the amph-fish, *A. kirkii,* sees out of water. Thus, the goal of the current research was to investigate the changes in the morphology of the eyes and structure of the retinal photoreceptors of the amph-fish *A. kirkii* and compare these adaptations with that of the mosquito fish *G. affinis* to comprehend the visual adaptation out of water in the amph-fish.

MATERIALS AND METHODS

1. Specimens collection

The materials for this study consisted of 42 specimens in total: 18 specimens of *A. kirkii* (Fig. 1), and 24 specimens of mosquito fish, *G. affinis* (Fig. 2). Samples of *A. kirkii* were gathered freshly in the summer of 2023 from Ras Mohamed's intertidal zone in Sharm El-Sheikh. The main method of fish collection was by hand. EL-Sayeda Aisha market provided live specimens of *G. affinis* in the summer of 2023. When feasible, fish were immediately inspected or stored in 10% formalin solution for a later inspection. The marine biology lab at the Zoology Department of the Faculty of Science at Al-Azhar University received the specimens of these two species. In the lab, fish were classified, to the greatest extent feasible, up to genera using the identification of **Bishai and Khalil (1997)** and **Lieske and Myers (2004)**. Then, the following experiments were conducted.

2. Eye shape

This study examined the relationship between eye diameter (ED) and axial length (AL) of the eye as the eye shape. The maximum (ED) and the maximum (AL) of each eyeball were measured to the nearest 0.1cm using digital calipers. These values were transformed to the logarithm ($\log_{10}$) values to calculate the eye shape ratio according to **Hall and Ross (2007),** as the following equation:

$$\textbf{Eye shape} = \log_{10} \{ED / AL\}$$

3. Histological investigations

The live specimens of *A. kirkii* and *G. affinis* were anesthetized. After dissecting the head, the eyes were removed, and corrected for at least 48 hours right away in alcoholic Bouin's fluid, dehydrated in ethyl alcohol rising concentrations, put in xylene, and dipped in wax (M.P.: 58°C). Perpendicular sections in eyes were cut by Technic Rotary Microtome stained with Harris's hematoxylin and eosin at thicknesses of 4-6μ **(Humason, 1979)** for regime histological studies. Lastly, the staining slides were inspected at various magnifications using a light microscope (XSZ-N107T), after which a digital camera (Toup Cam, Ver. 3.7) was used to take pictures.

4. Morphometric measurements of retina

Equipped with linear ocular micrometer, the thickness of the whole retina and its layers was determined in the fish under investigation. A method was employed to investigate the ratio of the relationship between the outer and inner nuclear layers according to **Wang *et al.* (2011)** and **Darwish *et al.* (2015)** to determine the size of the retina in eyes of the amph-fish *A. kirkii* and the non-amph-fish *G. affinis*.

RESULTS

1. Eye shape

The eyes of *A. kirkii* are bulging, lying in the upper position of the head, in addition the eyes set on retractable stalks on the head top (Fig. 1) permits the foraging of these fish on tidal flats. The eyes of *G. affinis* are without retractable stalks and they lie in the normal position on the head side (Fig. 2).

Fig. 1. A photograph of the Kirk's blenny, *Alticus kirkii* showing the eye is bulging, lies in upper position and on retractable stalks on the top of the head

Fig. 1. A photograph of the mosquito fish, *Gambusia affinis* showing the eye lie in normal position and without retractable stalks

Data in Table (1) explain that the ratio of eye diameter (ED) with head length of *A. kirkii* was lesser (35.77± 8.20cm) than that recorded in *G. affinis* (37.11 ± 1.28cm). While the the ratio of AL with head length (HL) had higher value in *A. kirkii* (40.73 ± 8.05) than those in *G. affinis* (39.24 ± 1.27). The eye shape showed slightly lower values in *A. kirkii* than that recorded in *G. affinis* (Table 1).

Table 1. Comparison in eye measurements and shape in relation to body measurements (mm) between amph-fish, *A. kirkii* and non-amph-fish, *G. affinis*

Measurement	*Alticus kirkii*		*Gambusia affinis*	
	Range	Average ± SD	Range	Average ± SD
TL (cm)	2.1 – 7.3	4.44 ± 1.14	2.71 – 3.47	3.11 ± 0.47
St.L (cm)	1.85 – 5.85	3.73 ± 00.94	2.11 – 2.89	2.47 ± 0.25
HL (cm)	0.27 – 1.1	0.65 ± 0.18	0.55 – 0.79	0.65 ± 0.08
HL/ St.L (%)	14.49 – 19.79	17.18 ± 0.93	21.93 - 30.27	26.44 ± 2.18
ED (cm)	0.12 – 0.30	0.20 ± 0.04	0.21 – 0.29	0.25 ± 0.03
ED/HL (%)	27.27 - 44.44	35.77± 8.20	36.71 - 38.18	37.11 ± 1.28
AL (cm)	0.14 – 0.33	0.23 ± 0.03	0.23 – 0.32	0.27 ± 0.03
AL/HL (%)	30 - 51.85	40.73 ± 8.05	37.97 – 40.51	39.24 ± 1.27
ED/AL (%)	108.33 – 118.75	110.45 ± 2.51	111.54 – 116.67	113.67 ± 1.58
Eye shape = $\log_{10}$ {ED / AL}	- 0.06: - 0.04	- 0.05 ± - 0.01	- 0.03: - 0.04	- 0.035 ± - 0.005

AL: Axial length of eye; **ED**: Eye diameter; **HL**: Head length; **TL**: Total length, **St.L**: Standard length

2. Histological structure of the eye

The general histological structure of the eyes in *A. kirkii* (Fig. 3) and *G. affinis* (Fig. 4) are like those of other vertebrates. The anterior and posterior chambers of the aqueous cavities, which are situated in front of the lens, and the big chamber of the vitreous body comprise the organization of the eyes. The anterior part of the outermost layer of the eye is transformed into a transparent cornea with the iris encircling the pupil, which is a central hole. The crooked internal layer of the cornea is very curled in *A. kirkii* which enables these fishes to graze on tidal flats, while reducing in *G. affinis*. The lens is entirely spherical and protrudes into the aqueous chamber. Moroever, the lens is more stable, slightly flattened and larger in *A. kirkii* than *G. affinis*. The middle layer of the eye is made up of choroid and a vascular tunic. The former structure is highly vascularized and situated between the protecting sclera and the photoreceptive. The light-sensitive area inside the choroid that vanishes close to the lens is caused by the photo-sensory cells found in the retina, which is made up of many layers. Rods and cones are the two main types of sensory cells found in the retina. The retina is more in thickness in *A. kirkii* than *G. affinis*. Lastly, the sclera, which is the posterior portion of the outermost layer and the site of the insertion of the ocular muscles, (Figs. 3 & 4).

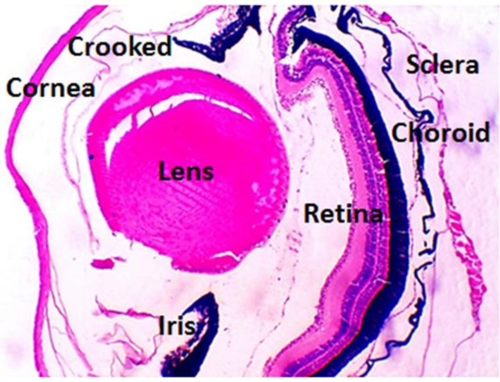	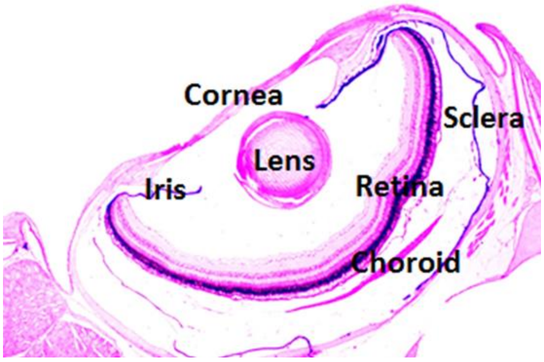
Fig. 3. A photomicrograph of V. S. in the eye of *Alticus kirkii* showing the general structure (H&E X 40)	**Fig. 4.** A photomicrograph of V. S. in the eye of *Gambusia affinis* showing the general structure (H&E X 40)

3. Histological structure of the retina

The retina's typical histological structure in *A. kirkii* (Fig. 5) and *G. affinis* (Fig. 6), comprises 10-layer neuronal retina on the inside and pigmented epithelium on the outside. The rod and cone layers are made up of inner and outer segments that are situated between the pigmented epithelium and the exterior limiting membrane. The retinal layers normally appear as follows: (1) Pigmented epithelium (PE) of simple columnar epithelial layer; (2) Visual elements "Rods and cones layer"; (3) External limiting membrane (ELM); (4) Outer nuclear layer (ONL); (5) Outer plexiform layer (OPL); (6) Inner nuclear layer (INL); (7) Inner plexiform layer (IPL); (8) Ganglion cell layer (GCL); (9) Nerve fiber layer (NFL) and (10) Inner limiting membrane (ILM) (basal lamina) (Figs. 5, 6).

The retina's outermost layer of *A. kirkii* (Figs. 5, 7) and *G. affinis* (Figs. 6, 8) have a deep brown color due to the inclusion of melanin granules. The exterior layer is comprised of a single layer of epithelial cells called the pigmented epithelium, which extends outer to the photoreceptor layer to support this layer as well as absorbing the scattered light passing through these cells (Figs. 5 - 8).

In *A. kirkii* (Figs. 5, 7), the results showed that the external layer of pigmented epithelium was distributed in the peripheral region of photoreceptors layer with a thick dense layer; while in *G. affinis* (Figs. 6, 8), it was distributed in epithelial cells inside peripheral region of photoreceptors layer with a less thickness layer covering the lowest part of the photoreceptor layer (Figs. 5 - 8).

In *A. kirkii* and *G. affinis*, the visual cell layer (VCL) is made from single and double cone types. In *G. affinis*, the cones are less abundant along this layer; while in *A. kirkii*, they are more abundant and much larger than those in *G. affinis* (Figs. 7 - 10).

In both *A. kirkii* and *G. affinis*, the outer limiting membrane appears as a clear light layer present in all areas, and the photoreceptor layer is separated from the external nuclear layer (ENL). The outer nuclear layer (ONL) consists of visual cell bodies, and its thickness varies between the two species. In *A. kirkii*, the number of cell rows ranges from 4 to 5 in a scattered pattern, while in *G. affinis*, the number of rows ranges from 3 to 4 in a more condensed arrangement (Figs. 7–10).

The outer plexiform layer (OPL) thickness in the two studied species varies from each other. In *A. kirkii*, the layer is narrow with densities of both bipolar and horizontal cells, while in *G. affinis*, this layer is wide and links with the axons of the visual cells and the densities of both bipolar and horizontal cells (Figs. 7 - 8).

The inner nuclear layer (INL) is distinguished by its heterogeneous cell composition. This layer is thick in the two species studied, being more crowded in *A. kirkii* than *G. affinis* (Figs. 7 - 8).

In both studied species, the thickness of the outer plexiform layer (OPL) is smaller than that of the inner plexiform layer (IPL). The inner plexiform layer, which makes up a single stratum of ganglion cells, is made up of bipolar cell axes and interlocking GC dendrites. Axons in the cell ganglion layer (GL) assemble to create the nerve fiber layer (NFL), which becomes denser as it approaches the optic nerve, which originates from the eye and ultimately connects to the brain. The thickness of the optic nerve fiber layer varies between the two species. It is an inner limiting membrane that divides the retina from the vitreous humor. It is a foundation plate made of Muller cells (Figs. 5 - 8).

Fig. 5. Enlarged portion of V. S. in the retina of *Alticus kirkii* showing layers of retina; **1**: Pigmented epithelium; **2**: Photoreceptor layer; **3**: Outer limiting membrane; **4**: Outer nuclear layer; **5**: Outer plexiform layer; **6**: Inner nuclear layer; **7**: Inner plexiform layer; **8**: Ganglion layer; **9**: Nerve fiber layer and **10**: Inner limiting membrane (H&E X 400)

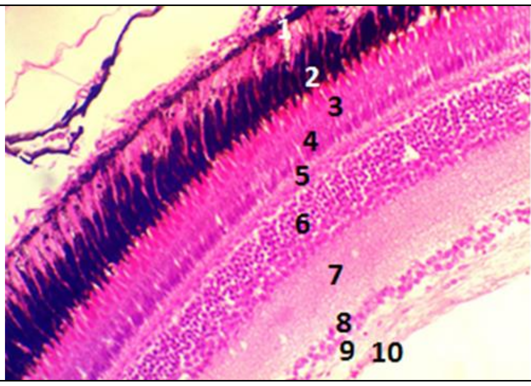

Fig. 6. Enlarged portion of V. S. in the retina of *Gambusia affinis* showing layers of retina; **1**: Pigmented epithelium; **2**: Photoreceptor layer; **3**: Outer limiting membrane; **4**: Outer nuclear layer; **5**: Outer plexiform layer; **6**: Inner nuclear layer; **7**: Inner plexiform layer; **8**: Ganglion layer; **9**: Nerve fiber layer and **10**: Inner limiting membrane (H&E X 400)

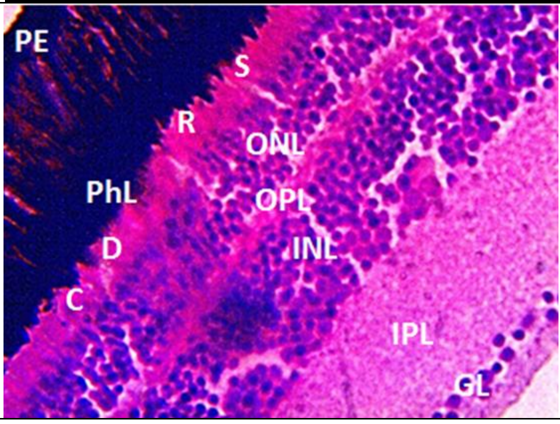

Fig. 7. Magnified portion of V. S. in the retina of eye *Alticus kirkii* showing pigmented epithelium (**PE**), photoreceptor layer (**PhL**) contain rods (**R**) and types cones (**C**); single (**S**) and double (**D**), outer nuclear layer (**ONL**), Outer plexiform layer (**OPL**), Inner nuclear layer (**INL**), Inner plexiform layer (**IPL**) and ganglion layer (**GL**) (H&E X 1000)

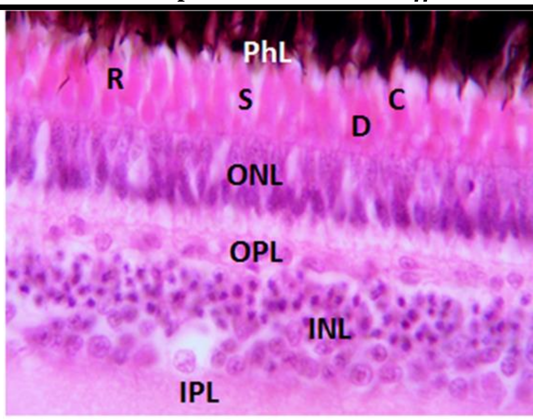

Fig. 7. Magnified portion of V. S. in the retina of eye *Gambusia affinis* showing photoreceptor layer (**PhL**) contain rods (**R**) and types cones (**C**); single (**S**) and double (**D**), outer nuclear layer (**ONL**), Outer plexiform layer (**OPL**), Inner nuclear layer (**INL**) and Inner plexiform layer (**IPL**) (H&E X 1000)

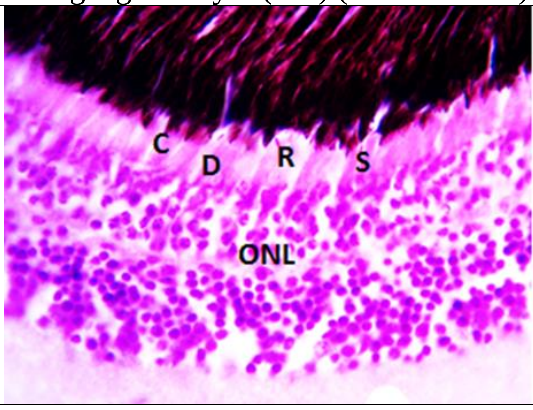

Fig. 9. Magnified portion of V.S. in the retina of eye *Alticus kirkii* showing rods (**R**) and types of cones (**C**); single (**S**) & double (**D**) and outer nuclear layer (**ONL**), (H&E X 1000)

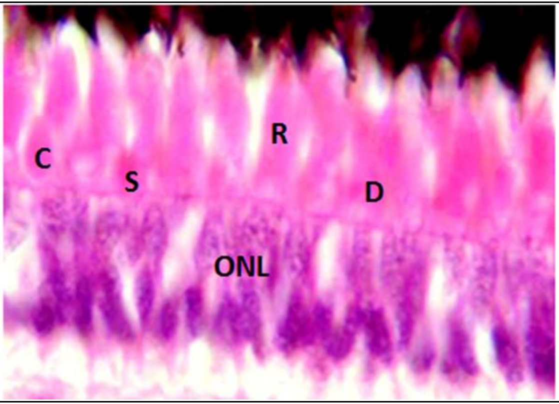

Fig. 10. Magnified portion of V. S. in the retina of eye *Gambusia affinis* showing rods (**R**) and types of cones (**C**); single (**S**) & double (**D**) and outer nuclear layer (**ONL**) (H&E X 4000)

4. Morphometric measurements of the retinal layers

There is a great variation that appeared between *A. kirkii* and *G. affinis* in the retinal layers' thickness. The results in Table (2) show that the retina is more in thickness in *A. kirkii* (530 ± 203.142um) than *G. affinis* (497.50 ± 140.60um)

The thickness ratio of pigmented epithelium layer is more in *A. kirkii* (15.20 ± 4.80%) than *G. affinis* (10.80 ± 1.50%) of the diameter of retina layer. The thickness ratio of photoreceptors layer is more in thickness in *G. affinis* (20.40 ± 3.60%) than *A. kirkii* (14.60 ± 1%) of the diameter of retina layer. The thickness ratio of the outer nuclear layer (ONL) in *A. kirkii* (9 ± 0.30%) is nearly like those in *G. affinis* (9.10 ± 1.90%) of the diameter of retina layer. While the thickness ratio of the outer plexiform layer is less in *A. kirkii* (3.20 ± 0.70%) than *G. affinis* (5.50 ± 1%) of the diameter of retina layer. The thickness ratio of the inner nuclear layer (INL) is less in *A. kirkii* (15.30 ± 2.30%) than in *G. affinis* (17 ± 1%) of the diameter of retina layer. The thickness ratio of the (IPL) is more in *A. kirkii* (22.90 ± 1%) than *G. affinis* (20.80 ± 1.80%) of the diameter of retina layer (Table 2).

Table 2. Comparison in thicknesses (%) of some neural retina layers between amph-fish, *A. kirkii* and non-amph-fish, *G. affinis*

Measurement (um)	***Alticus kirkii***		***Gambusia affinis***	
	Range	**Average ± SD**	**Range**	**Average ± SD**
RL (um)	320 – 800	530 ± 203.142	320 – 620	497.50 ± 140.60
PE /RL %	9.40 – 20	15.20 ± 4.80	9.40 – 12.90	10.80 ± 1.50
PhL/RL %	13.30 – 15.60	14.60 ± 1	15.60 – 24.20	20.40 ± 3.60
ONL/RL %	8.80 – 9.40	9 ± 0.30	6.70 – 11.30	9.10 ± 1.90
OPL/RL %	2.20 – 3.80	3.20 ± 0.70	4.40 – 6.50	5.50 ± 1
INL/RL %	12.50 – 18.20	15.30 ± 2.30	15.60 – 17.80	17 ± 1
IPL/RL %	21.90 – 23.80	22.90 ± 1	18.30 – 22.20	20.80 ± 1.80

RL: Retina layer; **PE:** Pigment epithelium; **PhL:** Photoreceptor layer; **ONL:** Outer nuclear layer; **OPL:** Outer plexiform layer; **INL:** Inner nuclear layer; **IPL:** Inner plexiform layer.

DISCUSSION

A unique class of fishes has evolved to view in the air **(Nicol, 1989)**. In this study, for attempt to understand how amph-fish, *A. kirkii* visualize out of water, comparative analysis of the retinal photoreceptor structure and eye morphological adaptations of amph-fish, *Alticus kirkii* and non-amph-fish, *Gambusia affinis* were done.

A. kirki and *G. affinis* are diurnal fishes, highly social, typically nourishment during the day; this shows that like trout, salmon and tilapia; they display a behavioral reply to light as a primary component influencing to feeding activity **(Toguyeni *et al*., 1997; Azab *et al*., 2017)**.

In the current study, the eyes of *A. kirkii* are bulging, lying in upper position of the head, in addition the eyes set on retractable stalks on the head top; permits the foraging of mudskippers fish on tidal flats and help in permit focusing out of water **(Brett, 1957)** against the eyes of *G. affinis*. Furthermore, the ratio of axial length to head length had higher value in *A. kirkii* than those in *G. affinis*. It can be inferred that *A. kirkii's* high axial length permits focusing away from water.

In the present study, the cornea is more curved; the lens is more stable, slightly flattened and larger in *A. kirkii* than *G. affinis*, allows *A. kirkii* to forage on tidal zone. These findings were detected by **Zander (1974)**. He concluded that *A. kirkii* is offered by the very thick cornea conjunctiva. Extension of the visual field in this species is achieved by protrusion of the eyes from the head, by recession of the dermal pigment, and by a relatively large lens which allows better perception of marginal rays. **Brett (1957)** stated that fishes need to be able to see in the air if they are to dwell at the water's surface or if they are periodically completely out of the water. The mudskippers (Periopthalmidae) have eyes that are ideally suited for aerial vision due to a sharply bent cornea and a somewhat flattened lens that allow for focusing out of water.

In the current study, the crooked internal layer of cornea is very curled in *A. kirkii* and reduced in *G. affinis*. The crooked layer in the eye of *A. kirkii* acts as a buffer against waves and environmental pressure on the eye area for protection of lens where enables these fishes to graze on tidal area in out of water.

In the present study, the lens is more stable, slightly flattened and large in *A. kirkii* adapted to high temperature in the Red Sea (Tropical region), while the lens of *G. affinis* is completely rounded and small adapted to low temperature in the northern lakes (Subtropical region). **Mcfall-Ngai and Horwitz (1990)** determined that an amph-fish, *A. kirkii* that inhabits the beaches of the Red Sea and has a relatively high body temperature range, which tolerates the temperature, is home to the more stable lens proteins among the teleosts. Certain proteins such a-crystalline found in these lenses are more heat stable in animals with high body temperatures. There was no correlation between the lenses' water content and thermal stability. *A. kirkii* fish, whose lenses contain less water than other fish, have structural proteins with a similar level of stability.

In the present study, between the photoreceptive retina and the protective sclera, there is a highly vascularized area called the choroid. Similar findings were detected by various authors **(Bone & Moore, 2008; Genten *et al.*, 2009)**. They reported that the layer located between the photoreceptive retina and the protective sclera, referred to as the choroid, is a densely vascularized area. It might have a coating of reflective guanine crystals called a tapetum lucidum, which improves visual sensitivity in low light by reflecting light back into the eye that the retina fails to absorb.

According to the current study, the sclera, which is where the ocular muscles insert, is in the posterior region of the outermost layer. **Herbert *et al*. (2003)** found that to preserve the delicate internal parts of the eye, the sclera, the outer covering of the eye, is reinforced.

In the present investigation, the histological examination of the retina in both *A. kirkii* and *G. affinis* confirmed prior studies by revealing a retinal structure comparable to that of other vertebrates **(Genten *et al.*, 2009; Azab *et al*., 2017; Cortesi *et al*., 2020)**. An internal neural layer and an exterior pigmented epithelium are the two primary layers that make up the retina. This further subdivision of the neural layer into nine different layers is compatible with **Rodieck (1998)** a fundamental explanation of the vertebrate retina's anatomy.

The present investigation unveiled that both *A. kirkii* and *G. affinis* exclusively consist of cone photoreceptors, a feature that is also observed in other teleost fishes **(Allison *et al*., 2010)**. This may suggest that they have adapted visually to their aquatic habitat. The external layer of pigmented epithelium in *A. kirkii* was distributed in the peripheral region of photoreceptors layer with a thick dense layer while in *G. affinis*, it was distributed of epithelial cells inside the peripheral region of photoreceptors layer with less thickness layer, where it covers a greater area of the photoreceptor layer in *A. kirkii*. This implies the existence of a species-specific adaptation, which may be associated with variations in their habitats or behaviors, as emphasized by **Cortesi *et al*. (2020)**.

This study demonstrated that the ratio of pigmented epithelium with retina layer had higher average value in *A. kirkii* (15.20 ± 4.80) than those in *G. affinis* (10.80 ± 1.50%**)**. This means that the range of vision in *A. kirkii* may be due to extending outside the aquatic environment. The visual adaptations to a thick pigmented epithelium in *A. kirkii* help in the protection of the rod's external segments. Similar results were detected in *Salmo gairdneri,* corroborating the importance of vision for teleosts **(El-Bakary, 2014)**.

Significantly, the photoreceptor layer in both diurnal species is composed of single and double cones which arranged in a square mosaic shape. These results agree with that obtained by **Fishelson *et al*. (2004)** for the diurnal cardinalfish (Apogonidae). Nevertheless, discernible distinctions can be observed in their configuration and dimensions between the two species. In the present study, the cones in *A. kirkii* are more condensed and much larger than those in *G. affinis*. Similarities in the configuration and dimensions of the cones may potentially indicate divergent color perception abilities and visual acuity among the species, as hypothesized by **Lythgoe and Partridge (1989)**.

The current investigation revealed that the thickness ratio of the outer nuclear layer (ONL) in *A. kirkii* (9.0 ± 0.30%) is nearly like those in *G. affinis* (9.10 ± 1.90%) of the retinal diameter. These may reflect the abundant increase of photoreceptors which reflected bio-activation of the vision **(Azab *et al*., 2017)**. **Dowling (1987)** noted that the purity of the outer limiting membrane is a trait that is characteristic of healthy retinal tissue in both species. The disparity in exterior nuclear layer thickness between the two species may serve as an indicator of variations in the density of the visual cell body, potentially associated with disparities in visual processing capacity.

In the current investigation, the thickness ratio of the outer plexiform layer and internal nuclear layer were less in *A. kirkii* than *G. affinis* of the diameter of retina layer, display a considerably greater density of bipolar and horizontal cells inside its outermost layer of *G. affinis*. As the hypothesis of **Marc and Cameron (2001)**, this difference may be indicative of distinct synaptic processing capacities in their retinas or due to variations in their visual processing pathways **(Dowling, 1987)**.

In the present investigation, the thickness ratio of the inner plexiform layer (IPL) is more in *A. kirkii* (22.90 ± 1%) than in *G. affinis* (20.80 ± 1.80%) regarding the diameter of the retina layer. This indicated that the linkage between bipolar and ganglion cells differs between species regarding the IPL and the ganglion cell layer (GCL). Additionally, the inner limiting membrane and nerve fiber layer thickness exhibited variation between the two species, which may suggest disparities in the transmission of signals from the retina to the brain between *A. kirkii* and *G. affinis*.

CONCLUSION

The eyes of *A. kirkii* are enlarged, positioned above, and attached to retractable stalks on the top of the skull; the cornea is more curved; the crooked is very curled; the lens is more stable, slightly flattened and large, allows *A. kirkii* to forage on tidal flats and help permit focusing out of water. Variations in photoreceptor arrangement and thickness of the retinal layers among the examined fishes may indicate divergent capacities for color perception and visual acuity, which may be associated with their unique ecological habitats.

REFERENCES

Alabssawy, A.N. and Khalaf-Allah, H.M.M. (2017). Comparative study on the adaptations for air-breathing and jumping in amph-fish, *Alticus kirkii* and non-amph-fish, *Gambusia affinis hollobrokii* Journal of American Science, **13**(12): 97–105.

Aljalaud, N.A. and Azab, A.M. (2021). Comparative studies on the histology of cornea and iris in eyes of a diurnal and a nocturnal Red Sea fish. EC Emergency Medicine and Critical Care, **5**(12): 144-153.

Allison, W.T.; Barthel, L.K.; Skedo, K.M.; Takechi, M.; Kawamura, S. and Raymond, P.A. (2010). Ontogeny of cone photoreceptor mosaics in zebra fish. Journal of Comparative Neurology, **518**: 4182-4195.

Azab, A.M.; Shoman, H.M.; El-Deeb, R.M.; Abdelhafez, H.M. and Abdel Samei, S.E. (2017). Comparative studies on the histology of eye retina in some Nile fishes with different dial activities. Egyptian Journal of Hospital Medicine, **68**: 815-823.

Bishai, H.M. and Khalil, M.T. (1997). Fresh Water Fishes of Egypt. Publications of National Biodiversity Unit, No. 9, Pp: 299.

Bone, Q. and Moore, R.H. (2008). Biology of Fishes, 3rd edition. Taylor & Francis Group, UK, Pp: 478**.**

Brett, J.R. (1957). The eye. In: The Physiology of Fishes. Brown, M.E. (ed.). New York: Academic Press, P. 121 – 154.

Brillet, C. (1986). Notes surle comportement du poisson amphibie *Lophalticus kirkii* Gunther (Pisces-Salariidae): Comparaison avec *Periophthalmus sobrinus* Eggert. Rev. Ecol. (Terre Vie)., **41**: 361-376.

Brown, C.R.; Gordon, M.S. and Chin, H.G. (1991). Field and laboratory observations on microhabitat selection in the amphibious Red Sea rockskipper fish, *Alticus kirkii* (Family Blenniidae). Marine Behaviour and Physiology, **19**: 1–13.

Brown, C.R.; Gordon, M.S. and Martin K.L.M. (1992). Aerial and aquatic oxygen uptake in the amphibious Red Sea rockskipper fish, *Alticus kirkii* (Family Blenniidae). Copiea, **4**: 1007–1013.

Cortesi, F.; Mitchell, L.J.; Tettamanti, V.; Fogg, L.G.; de Busserolles, F.; Cheney, K.L. and Marshall, N.J. (2020). Visual system diversity in coral reef fishes. In: Seminars in Cell & Developmental Biology Academic Press, Vol. 106: 31- 42.

Darwish, S.T.; Mohalal, M.E.; Helal, M.M. and El-Sayyad, H.I.H. (2015). Structural and functional analysis of ocular regions of five marine teleost fishes (*Hippocampus hippocampus, Sardina pilchardus*, *Gobiusniger*, *Mullus barbatus* and *Solea solea*). Egyptian J. of Basic and Applied Science, **1**: 8-17.

De Busserolles, F.; Fitzpatrick, J.L.; Paxton, J.R.; Marshall, N.J. and Collin, S.P. (2013). Eye-size variability in deep-sea lanternfishes (Myctophidae): an ecological and phylogenetic study. PLoS One, **8**(3): 1–14.

Dowling, J.E. (1987). The Retina: An Approachable Part of the Brain. Belknap Press.

El-Bakary, N.E.R. (2014). Visual adaptations of the eye of *Mugil cephalus* (flathead mullet). World Applied Sciences Journal, **30**(9): 1090-1094.

El-Bakary, N.E.R. and Abumandour, M.M.A. (2017). Visual adaptations of the eye of the gilthead seabream (*Sparus aurata*). Vet. Res. Commun., **41**: 257–262.

Fishelson, L.; Ayalon, G.; Zverdlng, A. and Holzman, R. (2004). Comparative morphology of the eye (with particular attention to the retina) in various species of cardinal fish (Apogonidae, Teleostei). The Anatomical Record, 277(A): 249-261.

Genten, F.; Trewinghe, E. and Danguy, A. (2009). Atlas of Fish Histology, Enfield (eds.), USA, Science Publishers., Pp: 215.

Gordon, M.S. (1998). African amph-fishes and the invasion of the land by the tetrapods. S. Afr. J. Zoology, **33**(2): 115-118.

Gordon, M.S.; Chin, H.G. and Martin, K.L.M. (1985). Aspects of the ecophysiology of terrestriality in *Alticus kirkii,* the rockskipper blenny of the Red Sea. American Zoologist, **25**(4): 123A.

Hall, M.I. and Ross, C.F. (2007). Eye shape and activity pattern in birds. Journal of Zoology, **271**: 437–444.

Herbert, N.A.; Macdonald, J.A.; Wells, R.M.G. and Davison, W. (2003). A difference in optomotor behaviour of two Antarctic nototheniid fishes is correlated with the presence of a choroids rete mirabile and Root effect. Polar Biol., **26**: 411–415.

Humason, G.L. (1979). Animal Tissue Techniques, fourth edition. Freeman, W.H. and company (eds.). San Francisco, Pp: 659.

Kassab, A.; Aoyama, M. and Sugita, S. (2001). The morphology of the iridocorneal angle of buffaloes (*Bos bubalis*): A light and scanning electron microscopic study. Okajimas Folia Anat. Jpn., **78**(4): 145–152 .

Klausewitz, W. (1964). Fische aus dem Roten Meer. VI. Taxonomische und okologische untersuchungen an einegen Fischarten der Kustenzone. Senck. Biol., **45**: 123-144.

Kondrashev, S.L. (2022). Photoreceptors, visual pigments and intra-retinal variability in spectral sensitivity in two species of smelts (Pisces: Osmeridae). Journal of Fish Biology, **101**(3): 584-596.

Land, M.F. and Nilsson, D.E. (2002). Animal Eyes. Oxford, Oxford University Press, Pp: 271.

Levine, J.S. and MacNichol, E.F. (1982). Color vision in fishes. Scientific American, **246**(2): 140-149.

Lieske, E. and Myers, R.F. (2004). Coral Reef Guide, Red Sea. Lieske, E. and Myers, R.F. (eds.). D and N publishing, Hungerford, Berkshire, Hong Kong., Pp: 365.

Lythgoe, J.N. and Partridge, J.C. (1989). Visual pigments and the acquisition of visual information. Journal of Experimental Biology, **146**(1): 1-20.

Marc, R.E. and Cameron, D. (2001). A molecular phenotype atlas of the zebra fish retina. Journal of Neurocytology, **30**(7): 593-654.

Mcfall-Ngai, M.J. and Horwitz, J. (1990). A comparative study of the thermal stability of the vertebrate eye lens: Antarctic Ice fish to the desert Iguana. Exp. Eye Res., **50**: 703-709.

Murdy, E.O. (1989). A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae: Oxudercinae). Records Australian Mus., Supp/'Il: 1-93.

Nicol, J.A.C. (1989). The Eyes Of Fishes. Clarendon, Oxford University Press, Pp: 328.

Oliver, F.J.; Samuelson, D.E. and Brooks, P.A. (2004). Comparative morphology of the tapetum lucidum (among selected species). Vet. Ophthalmol., **7**(1): 11–22.

Randall, J.E. (1983). Red Sea Reef Fish. Randall, J.E. (ed.). Hong Kong, Immel publishing limited. London WIX5AE, Pp: 192.

Rodieck, R.W. (1998). The first steps in seeing. Sinauer Associates is an imprint of Oxford University Press, Pp: 562.

Sayer, M.D.J. and Davenport (1991). Amph-fish: Why do they leave the water? Fish Biol. Fisheries., **1**: 159–181.

Schmitz, L. and Wainwright, P.C. (2011). Nocturnality constrains morphological and functional diversity in the eyes of reef fishes. Evol. Biol., **11**(1): 1–13.

Toguyeni, A.; Fauconneau, B.; Boujard, T.; Fostier, A.; Kuhn, E.; Mol, K. and Baroiller, J. (1997). Feeding behaviour and food utilization in *tilapia*, *Oreochromis niloticus*: effect of sex ratio and relationship with the endocrine status. Physiology and Behavior, **62**(2): 273–279.

Wang, F.Y.; Tang, M.Y. and Yan, H.Y. (2011). A comparative study on the visual adaptations of four species of moray eel. Vis. Res., **51**: 1099-1108.

Zander, C.D. (1974). Beziehungen zwischen korperbau und Lebensweise bei Blenniidae (Pisces) aus dem Roten Meer. III. Morphologie des Auges. Marine Biology, 28: 61–71.