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MONOCHROMATIC LIGHT AND PHOTORECEPTOR CELLS

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ABSTRACT

The present investigation is aimed to ascertain the movement of photoreceptor cells under different monochromatic light conditions. The under water vision depends on condition of water ie transparency of water, current of water, depth of water as well as on vision acuivity of species present there. Turbid and rough water may transmit less light than clear and clam water. In clam water species view the objects above the water through a circle above each eye, nearly all objects from horizon to horizon are viewed when window is enlarged with increasing depth. While the arial field is neither narrowed nor widened, there may be change in the brightness or distortion of objects with change is depth. Object appear largest when they are directly overhead. The present work reports the retinomotar responses to different monochromatic light conditions.

KEYWORDS : Monochromatic Light, Photoreceptor cells, Movement, Vision Eye

For visual perception of the environment fishes have well developed functional eyes. Underwater vision depends on condition of water (transparency of water, current of water, depth of water etc), as well as on vision acuity of fish present there. Turbid and rough water may transmit less light than clear and clam water there may be change in the brightness or distortion of objects with change in depth. Objects appear largest when they are directly overhead. The window is at its best when the fish looks directly upward and not slantingly upward at the water surface. Because the fish has no neck, the eyes protrude enough to be so located on the body as to give a full visual field. The position of eyes in fish provide a binocular vision in accordance with specialized habit of fish inspecting more restricted part of their surrounding. For proper vision wave-length and amount of light are important parameters (for detection of food and enemies), but for acute vision (resolution and sensitivity) the accommodation mechanism and retinal specialization, Nearly objects from large and brighter images than those far-away. Nearby objects demand accurate focussing by more precise adjustment. Thus, catching a prey less than one cm away of the snout would require a perfect accommodation of the lens in rostrad caudad direction. Such nearby vision would be sharp and myopic because of the high resolution in the caudad part of the retina. Retinal resolution on the other hand, is inversely related to separable angle and consequently proportional to density of photoreceptor and the size of eye. The structure of eye in fishes is quite similar although there are some differences in details, particularly with respect to visual cells in different species.

The retina has been the object of considerable investigation, because it is a readily accessible portion which facilitates the study of neurons, their structure and function. The structural organisation of the retina is highly ordered with the retinal elements arranged in relatively simple layers. The retina is made up of a thin outer and a thick innerwall. A space called ventricular space exist between the two. Thus the retina consists of the following layers which outside-inside is figure 1.

MATERIALS AND METHODS

Successful survival in any environment depends upon an organisms ability to acquire information from its environment through its senes. Fish have many of the same sense that we have, they can see, smell, touch, feel and taste, and they have developed some sense that we don't have such a electroreception. Fish cab sense light, chemical, vibrations & electricity.

Specimen were collected locally from fishing sites or procured directly from marked. These fishes (Live fishes) were found to be hardy withstanding in laboratory conditions and thus suitable for experimentation namely *C. batrachus*. The fishes were maintained in the aquarium under laboratory condition. A ready stock of 25 specimen were generally maintained. Live chironomid larvae earthworm bits or chopped goat liver were found to be readily acceptable food for these fishes.

Fish specimen were anaesthetized with 1:4000 aqueous solution of MS 222 and then examined under a zoom binocular with lateral side facing upward for eye. The eye was removed with care after cutting the optic nerve.

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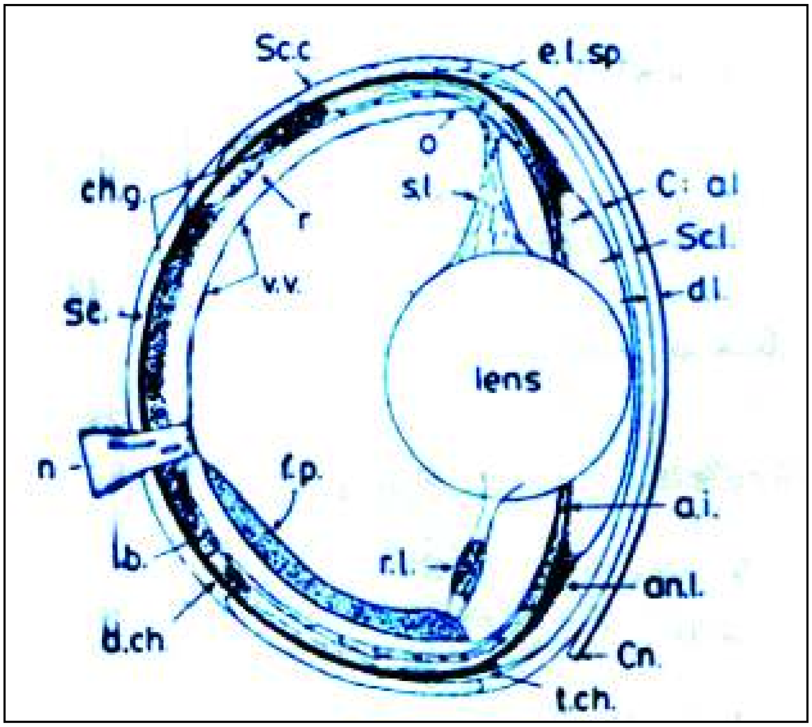

Figure 1 : Vertical Section of a Typical Teleost Eye

Removal of the lens was done at later stage during dehydration with 90% alcohal. A slit war made in the cornea to avoid damage to the retina during microtome sectioning. The section were cut 6 to 8 micron thick and stained with delafield heamatoxylin eosin and heidenhains Azam method Hunson (1972) the best result was obtained when section were given an overnight treatment with each mordant.

OBSERVATION

This topic includes the observations of various aspects of the retinal structure and their retraction under different light condition on *C. batrachus* and *L. rohita*. The experimental conditions consists of exposure of fishes to artificial conditions of monochromatic light red, blue, bluegreen. The major emphasis has been on the change of positions of photoreceptor cells and pigment epithelium layer, pigments of the retinal layer. The observations are based on microscopic examination of dorsoventrally sectioned eyes, cell counts in the retina measurement of different layers of retina and movement of photoreceptor cells. The internal structure of the retinal layers of catfishes have been earlier described by Verrier (1927-28), Wall (1942) and discussed by Singh and Munshi 1980 to which reader is referred for details. The present observations are focused on the nature of the photoreceptor cells and retinomotor responses under certain experimental monochromatic light conditions, particularly to establish whether they retreat up and down with respect to their normal position in normal light conditions (natural light).

***Clarias batrachus* (Lin.)**

Order	Cypriniformes
Suborder	Siluroidei
Family	Clariidae
Genus	*Clarias* (*Scopoli*)
Species	*C. batrachus* (*Linnaeus*)

Labeo rohita

Order	Cypriniformers
Division	Cyprini
Family	Cyprinidae
Genus	*Labeo* (*Cuvier*)
Species	*L. rohita* (*Hamilton Buchana*)

For monochromatic light, the procedure used for constant light illumination condition, was repeated with following alterations. A normal light was replaced by

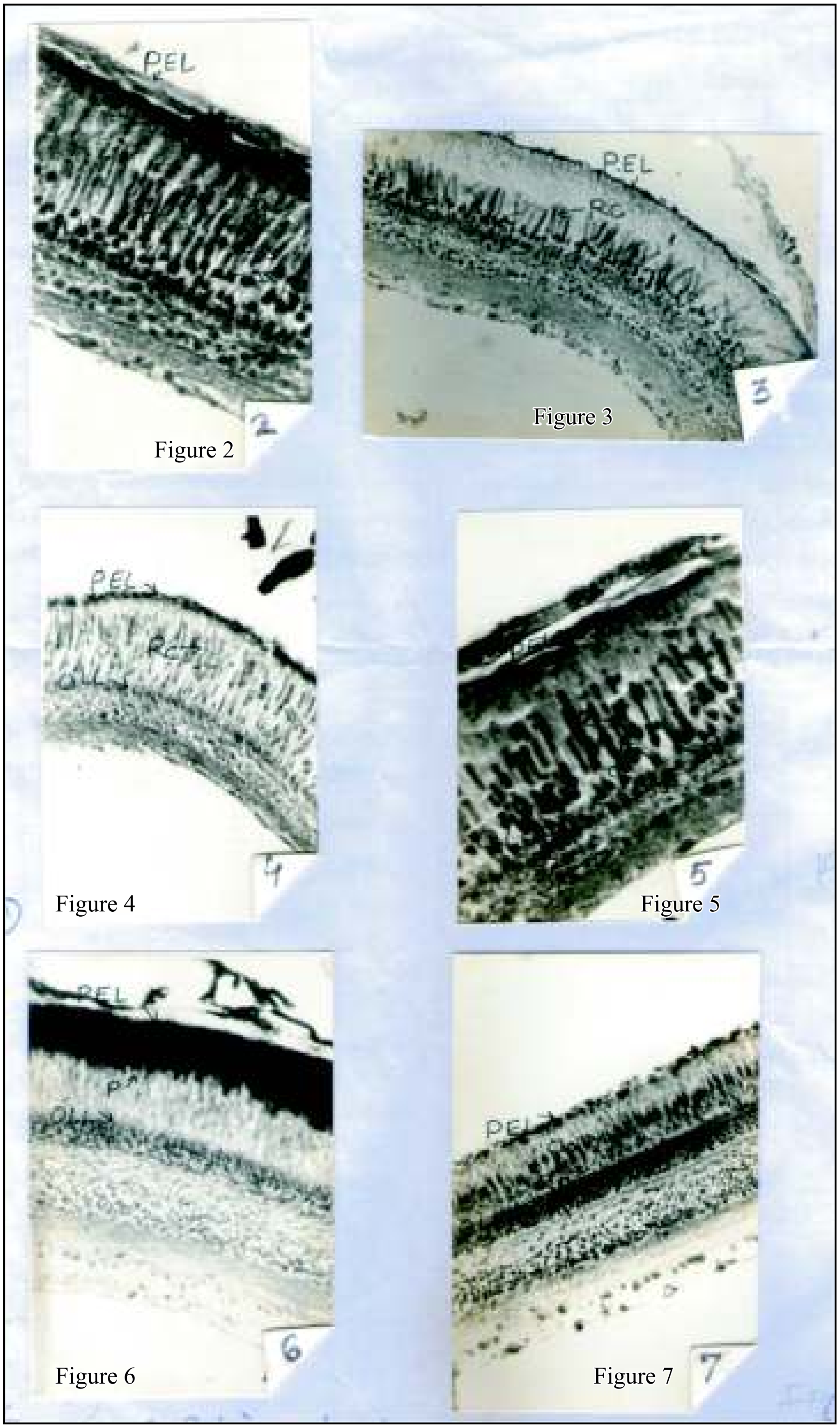

Figure 2 : Depigmented retina rods outer segments retreated up to outer limiting layer. Constant red light illumination. Azan X200.

Figure 3 : Depigmented retina constant green light illumination Azan X125

Figure 4 : Depigmented retina constant blue light illumination Azan X150

Figure 5 : Depigmented retina constant blue green illumination Azan X200

Figure 6 : Depigmented retina showing response to red light constant red light illumination Azan X200

Figure 7 : Depigmented retina, outer segment of rods contracted toward outer limiting layers and cones expanded away constant light illumination Azan X150

monochromatic light. The monochromatic light were red, blue green and blue-green. To obtain this coloured gelatin filter was used covering the bulb as well as the glass sides of the aquaria, the rest of the procedure were same as used for normal light condition.

The thickness of the retina as a whole or any of its component layers were measured slides. Alternatively, the thickness was also measured on the photomicrograph. These measurements served only to determine the ration in the thickness of one with the other. For measurement of actual cell size, oculometer and micrometer were used, wherever necessary.

Since the retina has curvature, hitological preparations pose problems in illucidating the presence of the two kinds of photoreceptor cells, the rods and cones under light microscope. Only electron microscope can convincingly demonstrate whether the photo receptor cells is rod or cone. However, present investigations revealed that retinomotor movement uder DD are ideal test to confirm the presence or absences of cones, because the two get. Distinctly separated out in two tiers distal cones and proximal rods.

On this basis, one can interpret easily the retinal organization with regard to photoreceptor cell type. On this basis in the present study, presence of cones could be revealed in *Labeo rohita* alone and absence of cones in the remaining fish examined and retnomotor responses under normal and cetain experimental light conditions particularly to establish whether they retreat up and down with respect to their normal position in normal conditions (natural light).

- Under constant red light condition (pigmented, partially depigmented and completely depigmented condition).
- Under constant green light condition (pigment, partially depigmented and completely depigmented condition).
- Under constant blue light condition (pigmented, partially depigmented and cmpletely depigmented conditions).
- Under constant blue, green light conditions pigmented partially depigmented and completely depigmented condition.

1. Red Light (About 700µm)

Exposure to red light evoke response in the rods. The outer segment remains in contracted position that is, close to outer limiting membrane. In this condition the response elicitated is similar to that shown by rods exposed to DD condition fig-2. The measurement of thickness of different layers retina of the fish examined are given below-

C. batrachus

(a)	1. Total thickness of retina	247.5µm
	2. 1 to 2 layer	150µm
	3. 1 to 4 layer	150µm
	4. 4 to 5 layers	26.25µm
	5. 8 to 9 layers	4.5µm
	6. 4 to 10 layers	97.5µm
	7. 6 to 8 layers	69µm

2. Green Light (About 525m)

Green light also evokes a change in the rods. The outer segments or the rods are contracted. The position of outer segments of rods is similar so that shown under red light condition. However, the cell count and active thickness of the various cell layer of the fishes examined are not the same fig-3 and the data on their measurement are given below.

C. batrachus

(a)	1. Total thickness of retina	240µm
	2. 1 to 2 layer	142µm
	3. 1 to 4 layer	150µm
	4. 4 to 5 layers	37.5µm
	5. 8 to 9 layers	3.75µm
	6. 4 to 10 layers	82.5µm
	7. 6 to 8 layers	52.5µm

Blue Light (About 400m)

Blue light also evokes a change in the rods. The outer segment of rates are contracted. however they do not come as close to the outer limiting membrane as well under red-light condition. It appears as if the contraction of outer segments or rods is not full fig-4. The measurement of the thickness of different layers of the retinal of the fishes examine are given below.

C. batrachus

(a)	1. Total thickness of retina	255µm
	2. 1 to 2 layer	112.5µm
	3. 1 to 4 layer	150µm

4. 4 to 5 layers	3.75μm
5. 8 to 9 layers	30μm
6. 4 to 10 layers	105μm
7. 6 to 8 layers	75μm

Blue Green Light (about 494μm)

At first glance, it appears that the change eliciated in rods is similar to that shown unde blue light condition, however when blue and green light effect fig-5 is compared with blue-green light effect. It is found that contraction of outer segments is less realized in the latter than in the former.

C. batrachus

(a)	1. Total thickness of retina	240μm
	2. 1 to 2 layer	112μm
	3. 1 to 4 layer	150μm
	4. 4 to 5 layers	30μm
	5. 8 to 9 layers	3.75μm
	6. 4 to 10 layers	105μm
	7. 6 to 8 layers	75μm

Labeo rohita

When exposed to constant red-light illumination condition, both types of photo-receptors, rods and cones show a response, whereas the outer segments of rods are contracted towards the outer limiting membrane, the outer segments or cones are expanded away from their outer limiting membrane, thus the response elicitated is opposite in the two and outer segment of the two occupy a position in tow tiers, an upper for cones and lower for rods.

The relative thickness or various cell layers were measured and the data are given below.

C. batrachus

(a)	1. Total thickness of retina	379.5μm
	2. 1 to 2 layer	142.5μm
	3. 1 to 4 layer	150μm
	4. 4 to 5 layers	82.5μm
	5. 8 to 9 layers	37.5μm
	6. 4 to 10 layers	247μm
	7. 6 to 8 layers	165μm

When only the thickness occupied by functional photosensitive segment (pigment epithelium cell and their process and outer segments of photoreceptor cells) is taken into account as to its relative development against the total thickness of the remaining retinal layers, it is found that greater in the two catfish species. It may be inferred that when layers 1 and 2 (pigment epithelium layer and photoreceptor cells layer) is considered in catfish species, the part of the retinal having neuronic elements inner to outer nuclear layer is less developed) than in salmonid, *Labeo rohita*.

When ration of the thickness of ganglion cell layer and never fiber layer to the total thickness of the retinal is taken into account, there is more or less constancy or values for these groups of fishes. When ration of the thickness of layers 4 and 5 (outer nuclear layer and outer plexiform layer) combine to layer 6, 7 and 8 (Inner nuclear layer, inner plexiform layer and layer of ganglion cells) is considered, it is found to be lesser in salmonid, *Labeo rohita* but greater in the catfish species indication is that the development of interneuronic connections are lesser developed in the catfish species than in salmonids, *Labeo rohita*.

From the above it is apparent that the retinal organisation in the catfish, *Clarias batrachus* examined presently deflates from those of the other teleosts examined, *Labeo rohita*. The latter have resemblence to the retinal organization found in typical normal teleost eye as that of salmonids, Al*,* 1971. The salmonids are known to be surface dwelling, fast swimming and diurnal forms which possess acute vision to rely upon in feeding and other activities. The fingerlings of *Labeo rohita* bear to salmonids is clear, because these are diurnal and surface dwelling forms, seemingly utilizing acute vision for feeding and other activities. However, catfish show a marked deviation from diurnal forms in the retinal organization. The two catfish species examined are known to be bottom dwelling and hiding by day, but surface dwelling and active by right. In accordance with this nocturnal way of life, vision apparently is of little use in feeding and other activities. These catfishes are known to possess ampullary electroreceptor besides ordinary neuromasts and taste buds (on barbles) on which these may be relying for feeding and other activities during night. In this background the elucidation of retinal components and their significance has remained an enigma. On the basis of present investigation and attempt is made to throw light on this issue. In terms of reduction in the relative thickness of the neural retina, the retinal, organization of presently examined catfishes *C. batrachus* is comparable with that of the bottom dwelling nocturnal glass catfish (non-indian) Wagner (1990).

2. In the teleosts namely *Labeo rohita* the retinal organization is similar to that of a normal day vision fish such as a salmonid. Fingerlings of *Labeo rohita* are surface feeder, Sahu and Jana (1996) and obviously have vision-dependent feeding.

The retina of these two teleosts display a typical columnar disposition. In *Labeo rohita*, photoreceptors include both rods and cones, which are easily distinguishable during retinomotor movements in response to total darkness, only single cones are discernible. Outer plexiform and inner plexiform layers are well developed. The ganglion cells to photoreceptor cell is normal i.e. 1:1.5.

It may be presumed that for these nocturnal catfish eyes are of no use in their nocturnal activities, feeding included. Vision dependent feeding if at all, may be taking place at sunset when these carnivorous fish are rising up and other diurnal fish are withdrawing from the surface. Silhouette/shadow overhead movement of the descending fish could be easily detected by the rods in the retina of these two catfishes as they are ascending the column of water for purpose of prey capturing.

It is interesting to note that literature on diurnal catfish retina is not available accept for the bottom dwelling (low illumination conditions Ameiurus nebulous, which, however, hunts in good light. The retinal of this catfish shows the presence of both rods and cones. In fact the retina of this fish is said to possess maximum numbers of cones (9000 per square mm) while the rods have numerically the least concentration (18400 per square mm) among the teleosts. Cone-high retina characteristic fish feeding in bright light, possessing good vision, such as in other vertebrates. According to Ali et al. (1961), sparseness of cones together with abundance of rods is characteristic of the retina of the vision-dependent fish. By comparison, the two nocturnal catfish species presently examined, show loss of cones, which can be said to be indicative of a degenerative changes.

Effect of Monochromatic light

Results of exposure to mono-chromatic light conditions in the case of four fishes examined are interesting and rewarding.

Underwater photic conditions particularly with regard to intensity of light and colour of light, varies with depth of water. Light a it penetrates the column of water from surface to bottom gets progressively attenuated owing to absorption as well as scattering of light. Behaviour of fish like of other organisms. Goldman and Horne, (1983) is strongly influenced by photic conditions available at habitat of fish, particularly in case of catfishes living at the bottom.

Ultra-violet and Infra-red components of light spectrum are absorbed fish and therefore penetrate least, Goldman and Horne, 1983. Blue and red light are attenuated very rapidly while yellow and orange light penetration are attenuated least.

Among the four fishes examined presently, *Labeo rohita* alone is diurnal with preponderance of retinal cones, the remaining three are nocturnal (bottom living and active by night). In the two catfishes and murrel, present histological investigation under light-microscope did not reveal the presence of cones.

As for as *Labeo rohita* is concerned, the red light evokes a retinomotor response similar to that produced by darkness, establishing high sensitivity of red sensitive cones. Spectral sensitivity curve for *Cyprinus carpio* retina shows that cone sensitivity for red is maximum at 620 mm. Akimichi and Tachibana, 1985.

In case of catfishes *Claries batrachus* and *Heteropneustes fossils*, red light and green light evoke retion-motor responses similar to one caused by darkness. Blue-green and blue light show slight but clear alteration in retinomotor responses as compared to those evoked by red and green light. Since rods are not known to be colour sensitive, presence of atleast blue-green and blue sensitive cones may be expected in their retina.

Colour vision does occur in teleosts which possess cones. According to the size and multiplicity, cones may be red sensitive, green sensitive, blue sensitive and ultraviolet sensitive, Stell and Harosi (1976), Wanger (1978), Wanger (1990).

The result therefore, calls for a review of cones controversy in catfishes. Electron microscopical examination, transmission and scanning, alone, may reveal the truth about the presence or absence of cones in the nocturnal catfish retina, if the retinomotor responses are not clear owing either to absence to cones or their inability to respond.

This fact could not be verified ye from experiments on retinomotor responses to constant light and darkness conditions. Indeed, catfishes are sensitive to some kind or colour vision, because by nature they are crepuscular, coming out of their hide at sun set. This demarks some dgree of colour sensitivity, particularly to twilight (dawn and dusk).

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