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[Research]

## Structure and arrangement of photoreceptors in the retina of big eye kilka, *Clupeonella grimmi* (Kessler 1877)

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### ABSTRACT

The big eye kilka, *Clupeonella grimmi*, is a marine fish living in depth of 20 to 200 meter of the Caspian Sea. Its eye and retina were processed for histological and SEM studies. Paraffin embedded retina was cut radially and tangentially in 5  $\mu$ m thickness and stained with hematoxylin and eosin method. The unstained sections were manipulated for SEM image observations. Tangential retinal sections showed irregular arrangement of cones. Five morphologically different types of photoreceptors were distinguished as rods, short single cones, long single cones, twin cones and double cones. The cones were counted in each quadrant of the retina and cone density was determined. The cones showed increment in diameter but reduction in number at anti-clockwise direction without any specific arrangement. Presence of four types of cone cells and their pattern of distribution revealed assistance in near surface color vision and more light capture in dim light of deeper waters as an adaptation to planktivorous feeding habit and deep water living habitat.

**Keywords:** retina, photoreceptors, *Clupeonella grimmi*, vision

### INTRODUCTION

The genus *Clupeonella* belongs to the family Clupeidae. Three species of the genus *Clupeonella* are found in the Caspian Sea. They are all small fish (8.5-14 cm) and commonly known as kilka (*Clupeonella cultriventris caspia* by Svetovidov, 1941), anchovy kilka (*C. engrauliformis* by Bordin, 1904) and big eye kilka (*C. grimmi* by Kessler, 1877) (Fazli et al., 2009). *Clupeonella* sp. is a commercially important fish in Iranian waters of the Caspian Sea as an important table fish, as prey for sturgeons, Caspian seal, Caspian perches, salmon and also for fish meal industry. *C. grimmi* is a small fish feeding mostly on zooplankton inhabiting the middle and southern part of the Caspian Sea at a depth of 20 to 200 m.

The aquatic environments possess a wide diversity of photic condition differing in many aspects such as color, clarity and turbidity (Bowmaker, 1995). A wide variety

of visual adaptations, particularly in eye structure and retinal cellular configuration found in teleosts are often related to their life habits and photic environment of the species (Reckel et al., 2003). Thus in many species regional differences occur within the retina, often resulting in considerable variations in photoreceptor types, densities and patterns (Reckel et al., 2003). Vision is the primary sense organ to fulfill the major behaviors, for example defense, feeding, and reproduction (Hanlon & Shashar, 2003). Presumably, both vision and lateral line are normally used to maintain school structure (Bone & Moore, 2008). Good vision increases foraging success and predator avoidance. The eyes of the most adult fishes are well-developed and chiefly designed for predator detection and prey location (Browman et al., 1990; Hawryshyn, 1997). Generally speaking, the retina of fishes has rod and cone cells. In teleost

fishes, the retina may contain several cone types that are morphologically different. Most of them are single or double cones but among them triple and quadruple cones are also observed (Engstrom, 1963). Rod cells are very sensitive to light, in contrast to cone cells which respond to intensive light (Kawamura & Tachibanaki, 2008). In most teleostean retina, the cones show mosaic arrangements (Lyll, 1957; Engstrom, 1963) but many species do not possess such patterns, which are rather irregular, as seen in nocturnal and deep-sea fish (Engstrom, 1963; Sandy & Blaxter, 1980; Braekevelt, 1984). To study the structure of retina and its cell arrangement, the species *C. grimmi* was selected as a pelagic marine fish living in large shoals and lacking lateral line.

#### Experimental Procedure

Fresh samples were obtained from local fishermen and Iranian Fisheries Department in Bandar Anzali, south of Caspian Sea. The specimens were fixed in Bouin's fluid and were transported to the laboratory of Biology, Faculty of Science, University of Guilan. After 24 hours, fish eye balls were dissected out, cornea and lens was removed gently. The left eye ball was divided into four quadrants namely: dorso-temporal (DT), ventro-temporal (VT), dorso-nasal (DN) and ventro-nasal (VN). The quadrants were transferred to 70% alcohol and after dehydration and clearing, were embedded in paraffin wax, radial and tangential 5 $\mu$  thick sections were made and stained with hematoxylin and eosin (H&E). The histological slides were studied and digital photographs were made. To exploit the details of events happening in histologically stained slides, paraffin sections were also prepared and treated to be scanned by Hitachi S-4160 SEM. A single desired unstained paraffin section was selected out of a serially sectioned retina and glued on a small 1x1

cm cover slip. The section was deparaffinized by drops of xylene and finally cleared by 90% alcohol for several times. It was then mounted on a stub, gold covered, scanned and micrographs were taken. Cone diameter and density in histological slides was calculated by Dinocapture version 3.3.016 software for each retinal quadrant.

#### RESULTS

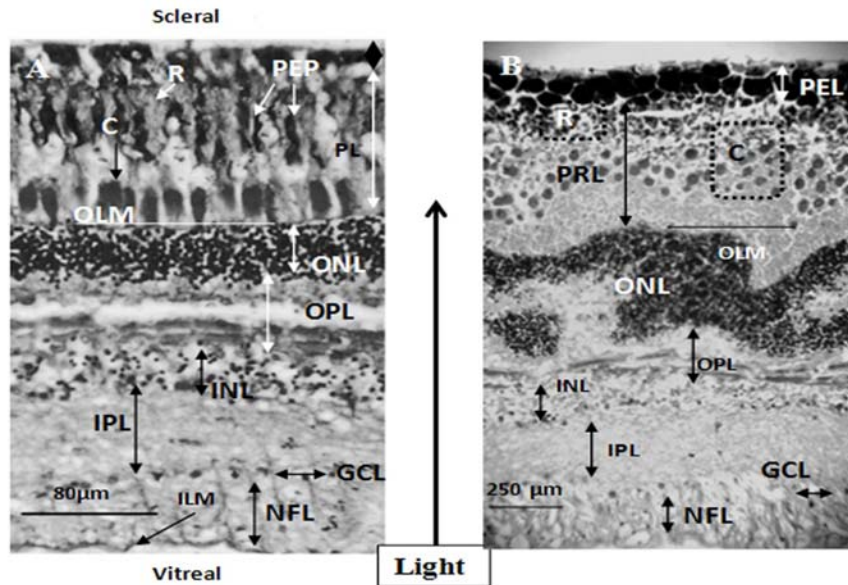
The eye retina of *C. grimmi*, like most vertebrates, is composed of ten layers namely, 1: Pigment epithelial layer (PEL), 2: Photoreceptor layer (PL) 3: Outer limiting membrane (OLM), 4: Outer nuclear layer (ONL), 5: Outer plexiform layer (OPL), 6: Inner nuclear layer (INL), 7: Inner plexiform layer (IPL), 8: Ganglion cell layer (GCL), 9: Nerve fiber layer (NFL), 10: Inner limiting membrane (ILM) (Fig.1, A&B).

The visual cell layer is composed of five morphologically different photoreceptor types: rod cells (Fig.2, B & C) short single cone cells, long single cone cells, twin cone cells and double cone cells (Fig 2, A-F). The rod cells are very large in number compared to cone cells.

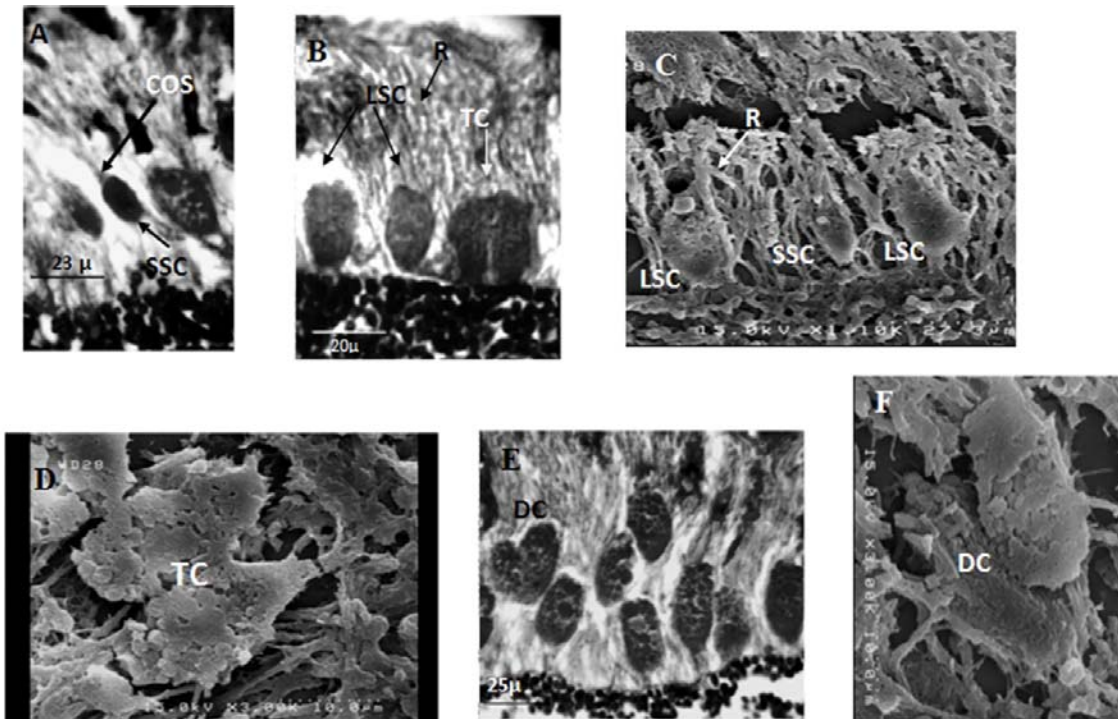
#### Cone Diameter And Density:

The least diameter of cones (all types) was found in VT quadrant and the largest cells were observed adjacent to VN quadrant (Fig.3) (Table 1). The highest cone density was observed in ventro-temporal quadrant and the ventro-nasal (VN) quadrant showed the least cone density (Fig.3) (Table 2).

The cones showed increment in diameter but reduction in number both at anticlockwise direction (Fig.3). The cone cells are large without any specific arrangement (Fig. 5A). Fig.5B is schematic image of cone non-pattern arrangements.



**Fig. 1:** Light microscope micrograph of radially (A) and tangentially (B) sectioned retina of *C. grimmii*, showing ten layers: PEL: pigment epithelial layer; PL: photoreceptor layer; OLM: outer limiting membrane; ONL: outer nuclear layer; OPL: outer plexiform layer; INL: inner nuclear layer; IPL: inner plexiform layer; GCL: ganglion cell layer; NFL: nerve fiber layer; ILM: inner limiting membrane; C: cone cells; R: Rod cells; PEP: pigment epithelial processes; PEL: pigment epithelial layer.



**Fig.2:** Retina of *C. grimmii*, micrographs of radial histological sections of retina- (A,B,E). SEM micrographs, radial sections of retina (C,D,F). SSC: Short single cone cell; COS: cone outer segment, LSC: Long single cone cell, R: Rod cell, TC: Twin cone cell, DC: Double cone cell.

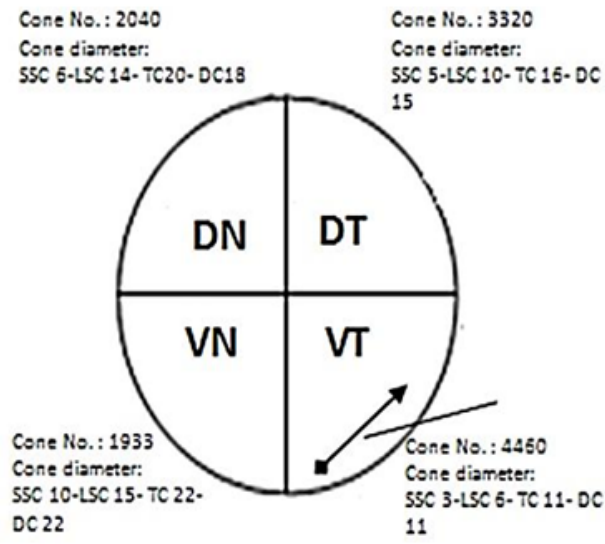


Fig.3: Left eye-Distribution pattern of cones. Number and diameter.

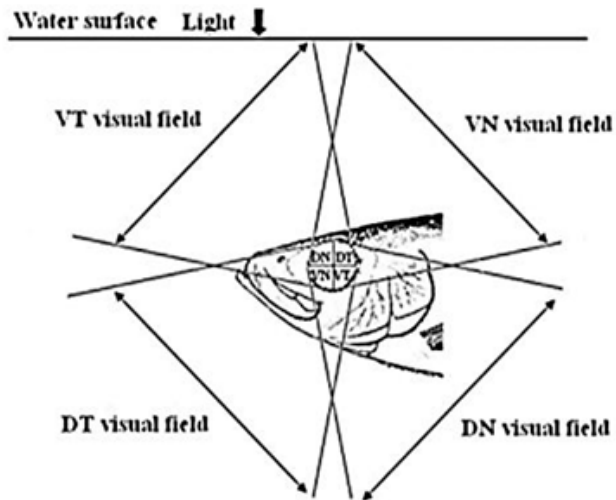


Fig.4: Visual field of each quadrant.

**Table 1:** Cone cell diameters in four retinal quadrants of *C. grimmi*.

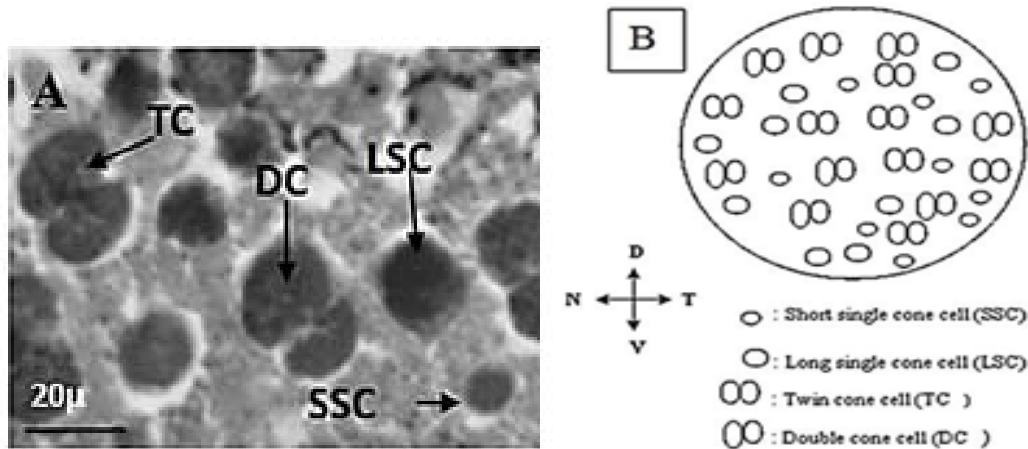
The type of cone cells	The cone cells diameter average in DT quadrant (mm) ± SD	The cone cells diameter average in VT quadrant (mm) ± SD	The cone cells diameter average in VN quadrant (mm) ± SD	The cone cells diameter average in DN quadrant (mm) ± SD
SSC	0.005 ± 0.0004	0.003 ± 0.0005	0.010 ± 0.0005	0.006 ± 0.0007
LSC	0.010 ± 0.0013	0.006 ± 0.0009	0.015 ± 0.0027	0.014 ± 0.0007
TC	0.016 ± 0.0028	0.011 ± 0.0005	0.022 ± 0.0013	0.020 ± 0.0016
DC	0.015 ± 0.0019	0.011 ± 0.000	0.022 ± 0.0013	0.018 ± 0.0005

SSC: Short single cone; LSC: Long single cone; TC: Twin cone; DC: Double cone; DT: Dorso-temporal; DN: Dorso-nasal; VN: Ventro-nasal; VT: Ventro-temporal; SD: standard deviation.

**Table 2:** Cone cell density in four retinal quadrants of the *C. grimmi*.

Positio	± SD	na (mm <sup>2</sup> )
DT	3320 ± 107	
VT	4460 ± 96	
VN	1933 ± 51	
DN	2040 ± 53	

DT: Dorso-temporal; DN: Dorso-nasal; VN: Ventro-nasal; VT: Ventro-temporal; SD: standard deviation



**Fig. 5:** (A): Tangential sections showing irregular cone arrangement in *C. grimmi* retina, TC: twin cone cell; DC: double cone cell; LSC: long single cone; SSC: short single cone. (B): schematic figure of cone non-pattern arrangement (D: dorsal. T: temporal. V: ventral. N: nasal).

**DISCUSSION**  
**Photoreceptor Types**

The eye of many fish contains both rod and cone photoreceptor cells that are

distributed throughout the retina (Fernald, 1988). Retina of deep-sea, fresh water, nocturnal fishes and fishes in twilight conditions have a large number of rod cells

(Wagner et al., 1998; Cowing et al., 2002). The cones are dominated in retina of fishes that inhabit photopic environments (Collin & Collin, 1988). In most retina, rods and cones differ in morphology, for example rods are expected to contain much more visual pigments than cones and are much more sensitive. Thus cones need higher light intensities to be stimulated. Chondrichthyes such as *Centrophorus*, chimaerids, rays, and some deep-sea teleosts have a pure rod composed retina. Usually, in fish that live in dim light, cone cells are absent (Bone & Moore, 2008). In *C. grimmi* both rod and cone photoreceptor cells are found. It is assumed that in this fish the presence of rod cells is an adaptation for dim light vision in deep waters and that of cone cells in *C. grimmi* are chiefly an aid for daylight vision in upper water layers.

#### Types of Cone Cells

The existence of three morphological types of cone photoreceptors i.e. single, double, and twin - has been well established from a host of classical anatomical studies of vertebrate retinas (Stell, 1972). Cone cells are morphologically different and in many cases offer various spectral sensitivity (Levine & MacNichol, 1979). Single cone cells (SC) have sensitivity to three distinct wave length groups centered around 360, 420, and 460nm (UV, violet, and blue) (Marshall & Vorobyev, 2003). Presumably, long single cone cells in *C. grimmi* occupy a predominant region of under 460 nm spectrum. Due to dim light of the deep water where *C. grimmi* lives it can also be suggested that the vast surface area of long single cone cells may trap more of light photons.

In many cases, short single cones have often been reported to be UV-sensitive (Bowmaker, 1984; Bowmaker et al., 1991; Novales & Harosi, 2000). UV vision is thought to play a role in intraspecific communication or navigation and also increasing the detection of prey (Browman et al., 1994; Losey et al., 1999) and an aid

to planktivorous fishes (McFarland & Loew, 1994).

Certainly, UV photons exist in clear water to a depth of at least 100 m (Losey et al., 1999). However, it is unknown if in the turbid waters of the Caspian sea UV-light penetrates deep enough in sufficient intensities to reach potential short single cones of the big eye kilka, therefore it is most probable that UV-sensitivity may assist plankton feeding habit of this fish merely in upper water layers.

Two members of twin cone cells are identical and fused throughout the length of the inner segment. They are usually found in teleost fish that exhibit a very effective preference for dim environment, therefore suggesting that twin cone cells may have some special properties for mediating dim light vision (Burkhardt, 1986). A controversial debate argues that the majority of twin cones are green sensitive, with few red-sensitive cells (Kusmic & Gualtieri, 2000). Considering the feeding habits of *C. grimmi* in intensified light, the later suggestion is most probable.

A double cone cell possesses one chief member and one accessory member (Walls, 1942). It is suggested that double cone cells are involved in color vision particularly red-green (Kusmic & Gualtieri, 2000) or blue-green retina of trout (Kusmic et al., 1993). The ability to discriminate between different wavelengths of light via photoreceptor types differing in peak wavelength sensitivity mean color vision (Marks, 1965; Stell & Harosi, 1976; Dowling, 1987). The presence of four types of cone cells in *C. grimmi* indicates that presumably these cones assist in color vision for fish in low depths and lead to better resolution of surroundings in dim light of deeper waters.

#### Cone Cell Patterns

Mosaic arrangement of cones is a characteristic feature of most teleost retina. Mosaic cone arrangement is found in two fundamental patterns namely square mosaic and row patterns. Square mosaic

pattern help shallow water diurnal fishes to trace moving objects in intensive light whereas deep sea crepuscular fishes possess row pattern cone arrangement assisting vision in dim or no light conditions with lower acuity (Boehlert, 1978; Rossetto & Sazima, 1992).

*C. grimmi* being a planktivorous at upper layers of water where light is present, was expected to have a row pattern of cone cells but as it is shown in Fig.5a and b retina of examined fish did not exhibit any forms of regularity and therefore it showed further similarity to retina of fishes inhabiting deep and dark environments (Engstrom, 1963; Sandy & Blaxter, 1980; Braekevelt, 1984; Braekevelt, 1994).

In this work, tangential sections of the retina showed sparsely distributed short single, long single, double, twin cone cells. Big eye kilka inhabits the relatively deeper areas of the central and southern Caspian Sea with distinctive adaptation to greater depths such as larger eyes, structural differences in their retinas and greater transparency of the tissues (Prikhod'ko, 1981). The presence of sparsely arrangement of all four types of cone cells justify the depth of below 70 meters where *C. grimmi* lives.

### Cone Cell Density

A useful indicator of visual acuity in teleost fish is cone density (Nag & Bhattacharjee, 2002). Usually, diurnal teleost fishes have cone density over 40000 per mm<sup>2</sup> (MacFarland & Munz, 1975; Ali & Anctil, 1976). In guppy and puffer fish (Tetraodontidae), this value exceeds 50000 per mm<sup>2</sup> (Muller, 1952; Ali & Anctil, 1976). The cone density average values of four quadrants of retina in the *C. grimmi* eye showed difference from 1933 to 4460 at ventro-nasal and ventro-temporal respectively (Table 2). Rod and cone cell diameters are larger in dorsal retina of *Osmerus eperlanus* that are looking towards a lower light intensities and increases the area per cell for photon capture. Also in the same fish the cone diameters are smaller in ventro-temporal area that is looking antero-

dorsally and enhances spatial resolution (Reckel et al., 2003).

The ventro-temporal area receives information from dorso-nasal part of the visual field that is of particular importance for many visually guided predators (Ahlbert, 1969; Zaunreiter et al., 1991; Rossetto, et al., 1992; Novales & Harosi, 2000; Reckel et al., 2001). The ventro-temporal area proposes acute vision in the upper frontal field of view corresponding with demands of the general forward orientation of teleost feeding and swimming (Heß, 2009).

In *C. grimmi* the highest cone density having smallest diameter was found in ventro-temporal region which as mentioned above refers to the demands of the general forward orientation of the feeding and swimming of this fish as a pelagic and zooplanktivorous fish and covers antero-dorsal visual field and enhances spatial resolution. The lowest cone density with largest cone diameter was observed in ventro-nasal region that help to receive more light and dim vision (Table 1 and Fig.3). The larger number of cone cells bring about acuity which happens at higher light intensities (VT quadrant) in the span of visual field. Instead in VN quadrant lower number of cone cells with larger cell size though not much effective to attain acuity but help in dim light vision. The cone density values in big eye kilka showed an anti-clockwise increment order from VT to VN (Fig.4).

### ACKNOWLEDGMENTS

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## ساختار و ترتیب سلولی دریافت کننده های نوری شبکه کیکای چشم بزرگ

### *Clupeonella grimmi* (Kessler 1877)

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(تاریخ دریافت: 92/5/24 - تاریخ پذیرش: 92/8/18)

#### چکیده

کیکای چشم درشت (*Clupeonella grimmi*) ماهی آب شور است که در اعماق 20 تا 200 متر دریای خزر یافته میشود. چشم و شبکه ماهی جهت مطالعات بافتی و میکروسکوپ روبشی آماده شد. شبکه احاطه شده در پارافین در جهات شعاعی و مورب با ضخامت 5 میکرون مقطع برداری شده و با روش هماتوکسیلین و ائوزین رنگ آمیزی شد. مقاطع بافتی رنگ نشده برای تصویر برداری با میکروسکوپ روبشی آماده سازی شد. مقاطع مورب ترتیب نامنظم مخروطی ها را نشان داد. پنج نوع دریافت کننده نوری که از نظر ریخت شناختی متفاوت بودند بصورت سلولهای استوانه ای، مخروطی منفرد کوتاه، مخروطی منفرد بلند، مخروطی دوگانه و مخروطی دوتایی شناسایی شدند. سلولهای مخروطی در هر ربع شبکه شمارش شدند و تراکم آنها تعیین شد. سلولهای مخروطی بدون هیچگونه ترتیب خاص قرار گیری دارای افزایش قطر ولی کاهش تعداد در خلاف جهت عقربه های ساعت بودند. وجود چهار نوع سلول مخروطی و نحوه پراکنش آنها نشان دهنده کمک به دید رنگی نزدیک سطح و دریافت نور بیشتر در تاریکی آبهای عمیق است و سازشی نسبت به عادت پلانکتون خواری و شرایط زندگی در آبهای عمیق می باشد.

\*- مولف مسئول