Comparative Light Microscopic Studies on the Retina of Some Elasmobranch Fishes

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Retinas of 11 species of elasmobranchs from 6 families including deep sea forms were examined by light microscopy. Variations in retinal structure were correlated with depth of capture. The retinas of shallow water forms had a well developed pigment epithelium, cone photoreceptors, numerous short rod outer segments and large horizontal cells with two or three layers. The retinas of deep sea species are characterized by possessing a reduced pigment epithelium, cone-free retinas and their rods have comparatively longer outer segments. No clear identification of bipolar, horizontal and amacrine cells was possible, but there are certainly no highly differentiated horizontal cells of the kind found in most shallow water fish retinas. However, the retinal structures of elasmobranchs are rather standardized and the dramatic variations of retinal structures which often occur in teleosts were not found in this observation.

Fishes live in more varied light environments than any other group of vertebrates. Such different environments must present widely different visual problems to their inhabitants.

A large amount of information concerning the microscopic structure of the retina has accumulated as a result of classical histological investigations. Most of the studies published were made on the limited taxonomic groups or ecological situations. Wunder1) was the first investigator to make tentative correlations between the numbers and characteristics of rods and cones, on the one hand, and the photic habitat, on the other. Studying on 24 species of fishes from different environments, Wunder1) made detailed measurements of sizes and numbers of visual cells. McEwan2) compared mormyrid retinas with those of some freshwater and marine, pelagic Isospondyli forms. Brauer (cited in Ali and Hanyu,3) and Munk4), pioneered research on deep sea teleost eyes, using nearly 100 species. Afterward this type of work has been sporadic, and only one or two species were used as research materials until Munk4) described the eyes of 37 species and reviewed previous works. A comparative study on the retinas of many deep sea teleosts was also carried out by Ali and Hanyu.3)

Similar histological works are seen on elasmobranchs, however emphasis had been placed on coastal and pelagic species with little attention on deep sea forms.5) Deep sea makes a biologically unique environment and elasmobranchs living there are useful research materials to examine retinal adaptation in comparison with teleost fishes.

The present light microscopic study aimed to supply additional comparative information on the morphology of the retina of several species of elasmobranchs belonging to various families. The structural variations in fish retinas relating to the depth of habitat and their functional significances are the major themes of this paper.

Materials and Methods

With a few exceptions, all specimens were chosen from catches using bottom or midwater long lines and surface drift net. Some specimens were obtained from a private aquarium. The names of families, species, range of depth of habitats, actual depths and locations of capture are given in Table 1.

The eyes were usually enucleated on board a ship or in the laboratory of aquaria. The retina was cut, together with the sclera, and fixed in 2% glutaraldehyde and 2.3% paraformaldehyde.

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<table>
<thead>
<tr>
<th>Family</th>
<th>Species</th>
<th>Total length (cm)</th>
<th>General range of habitat depth (m)</th>
<th>References</th>
<th>Captured at:</th>
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<td></td>
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<td>100-200 (92%)*2</td>
<td>Mikawa*3</td>
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<td>300-699</td>
<td>Kobayashi et al.*10</td>
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*1 The range of main fishing depth for Carcharhinus longimanus
*2 The range of main fishing depth for Mustelus manazo.
*3 The range of main fishing depth for Centroscyllium ritteri.
*4 The range of main fishing depth for Scymnodon squamulosus.
*5 The range of main fishing depth for Centrophorus acus.
buffered with 0.1 M phosphate. The specimens were then postfixed in 1.5% osmium tetroxide buffered with the same solution. The fixed retinas were dehydrated and embedded in epoxy resin. Semithin (1 μm) transverse and tangential sections were made with glass knives on a Sorvall ultramicrotome (MT-1) and stained with toluidine blue.

The dimensions of different cellular components were measured on enlarged photographs of these transverse sectioned preparations. Measurements of the following were made: 1) retinal pigment epithelium, 2) rod outer segment, 3) rod inner segment and outer limiting membrane, 4) outer nuclear layer and outer plexiform layer, 5) inner nuclear layer, 6) inner plexiform layer, ganglion cell layer, nerve fiber layer and inner limiting membrane. In most of the fishes, retinal thickness varied considerably according to the retinal region examined. Therefore, comparison among the species of fish used was made with measurements in five regions near the fundus. The diameters of rod outer segments were measured on enlarged photographs of tangential sections. These data are given in Table 2, in which the thickness of each layer comprising the retina is expressed as a percentage of the entire retina for comparison.

**Results**

The Selachians are divided into two orders, Euselachii and Batoidai, but the eyes differ little between them. The former comprising the sharks is an older group of fusiform shaped fishes and the latter is modified group with flattened body and includes skates, rays and torpedoes.

**Euselachian Fishes**

It is clear from the examination of transverse sections (Fig. 1) and Table 2 that the retinal structure in carcharhinid and triakid sharks is relatively similar to each other. Although the pigment epithelium, which forms the outermost part of the retina, is apparently well developed, it
Fig. 2. Optical micrograph of transverse section through the retinas of some euselachian (scyliorhinid and squalid) fishes: a, Deania eglantina; b, Apristurus macrorhynchus; c, Etmopterus lucifer; d, Scymnodon obscurus; e, Centrophorus tessellatus. The inset in Fig. 2a shows a cone-like cell of D. eglantina. Bars indicate 20 μm.
Fig. 3. Optical micrograph of transverse section through the retinas of some batoid fishes: a, Dasyatis akajei; b, Dasyatis kuhlii; c, Urolophus aurantiacus; d, Platyrhina sinensis. The inset in Fig. 3c shows a cone-like cell of U. aurantiacus. (d) is taken from a paraffin section (5 μm). Mallory’s triple stain. Bars indicate 20 μm.
seems to be devoid of pigment. In both specimens, the overwhelming majority of photoreceptors are the slender, cylindrical rods with outer segments which extend into the pigment epithelium. The average length of the rod outer segments was 41.4 μm in *P. glauca* and 28.4 μm in *T. scyllia*. The diameters averaged 2.4–2.6 μm for the outer segments.

In contrast to rods, the cones were short and pyramidal in shape and their outer segments did not extend as far as the pigment epithelium (Fig. 1, insets). Currently proceeding work shows that at least two other sharks (*Carcharhinus longimanus* and *C. plumbeus*) possess cones which are morphologically similar to those of *P. glauca*.

The nuclei of rods as well as cones were located within the outer nuclear layer: cone nuclei were indistinguishable from those of rods. The outer nuclear layer is made up of about three to four rows of small, uniform, well-stained nuclei. This layer is thinner than the inner nuclear layer, the former accounts for 14.3–17.2% of the total retinal thickness and the latter 22.8–23.3% (Table 2). The latter layer is composed of two or three layers of large horizontal cells, a few bipolar and amacrine cells. The ganglion cell layer is composed of sparsely distributed large cells. The nerve fibers from the ganglion cells form a loosely packed fibrous layer.

The retinal structures of scyliorhinid and squalid sharks are shown in Fig. 2. The pigment epithelium is usually thin and is in the form of a narrow strip. The photoreceptor cells, which are a uniform population of rods, have a cylindrical form throughout their length from the tip to the base of the outer segment, and vary little in diameter in their different regions. They ranged in length from 39.9 μm in *E. lucifer* to 50.6 μm in *C. tessellatus*, and in width from 3.7 μm in *S. obscures* to 4.1 μm in *C. tessellatus*.

A few cone-like cells have been found to date among the hundreds of rods in case of the retina of *D. eglantina* (Fig. 2a, inset). The other species employed in this paper, however, seem to have a cone-free retina.

In accordance with the larger number of rods, the outer nuclear layers are also thicker, being made up of four to seven rows of well-stained nuclei. The inner nuclear layer contains the nuclei of bipolar, horizontal, amacrine and Müller cells. In most species the horizontal and bipolar cells lie immediately adjacent to each other, and in some are indistinguishable by light microscopy. The other layers of the retina are essentially similar to those of the species mentioned above.

**Batoid Fishes**

The main features of 4 species of rays are shown in Fig. 3. The thickness of pigment epithelia varies from a minimum of 10.0 μm in *Urolophus auranticus* to 15.0 μm in *Dasyatis kuhlii*. Their nuclei are almost spherical and detached fragments of rod outer segments are observed sporadically in their cytoplasm. The rod outer segments are characteristically stained in heavy color, while the ellipsoid and myoid stained in homogeneous light blue color. The outer segments are large and cylindrical in shape, and extended into the pigment epithelium. The average length of the rod outer segments ranged from 29.1 μm in *D. akajei* to 33.9 μm in *D. kuhlii*. The diameters of them ranged from 3.2 μm in *D. kuhlii* to 5.1 μm in *Platyrhina sinensis*.

Except for *P. sinensis* cones were present in the batoid fishes studied. The cone nucleus occurs on the external limiting membrane. The cones in *Urolophus auranticus*, however, are not detectable as evident. To date only two cone-like cells have been found in *Urolophus* retina among a lot of preparations from the six eyes (Fig. 3c, inset).

The outer nuclear layers are composed of 2–4 tiers of rod and cone nuclei. The outer nuclear layers are thinner than the inner layers (Table 2). The figures vary between 10.6 and 17.2% in outer nuclear layers and those of the latter layers are 20.7–24.7%. The inner nuclear layer contains the nuclei of bipolar, horizontal, amacrine, and Müller cells. In general, horizontal cells are large and are arranged in two or three layers. The outer horizontal cells are especially large and cuboidal. The intermediate or inner horizontal cells are seen on the vitreous side. Sparsely arranged ganglion cells form an ill-defined layer. The nerve fibers run between and in proximity to the ganglion cells forming a thin but separate layer.

**Discussion**

The pigment epithelium in elasmobranch retinas varies greatly in its development, being prominent in shallow water forms, whereas it is less prominent in deep sea species. In many deep-dwelling teleosts, similar instances were reported by Brauer (cited in Ali and Hanyu,3) and Munk4). In deep water teleosts, the pigment epithelium is weakly developed, without
Table 2. Measurements of retinal elements in the fishes studied*

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<thead>
<tr>
<th>Species</th>
<th>Entire retina (thickness)</th>
<th>RPE (thickness)</th>
<th>Rods</th>
<th>ONL-OPL (thickness)</th>
<th>INL (thickness)</th>
<th>IPL-ILM (thickness)</th>
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<td>*P. glauca</td>
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<td>17-20 (18.1)</td>
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<td>Rat. (%)</td>
<td>100</td>
<td>6-8 (6.7)</td>
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<td>8-10 (9.2)</td>
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<td>193-205 (200.6)</td>
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<td>Rat. (%)</td>
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<td>2-3 (2.5)</td>
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* The figures in the parenthesis represent mean.

Abbreviations: RPE=retinal pigment epithelium; ROS=rod outer segment; RIS=rod inner segment; ONL=outer nuclear layer; OPL=outer plexiform layer; INL=inner nuclear layer; IPL=inner plexiform layer; ILM=inner limiting membrane.
extensive processes protruding between the outer segments.

A direct relationship was found between the average length of outer segments and the ranges of living depth in the species studied (Table 2). There was a tendency that the rods in T. scyllia collected from shallow depths were shorter and more slender than those of the other sharks in the present study. In sharks from waters deeper than 400 meters which are occasionally caught from lesser depth, the outer segments are slightly longer, measuring 39.9–50.6 μm in length and 2.6–4.1 μm in diameter. The length of rod outer segments of batoid fishes appears to be intermediate between shallow and deep sea forms (length of the rod outer segments, 29.1–33.9 μm; diameter of the rod outer segments, 3.7–5.1 μm).

The length of rod outer segment in our result is slightly longer than that in other investigators9–13, since they used paraffin embedded specimens but we fixed the retina by the aldehyde and the osmium fixatives and embedded them in epon plastic. The differences in histological methods may have produced different shrinkage rate of tissue. However, the tendency and figures in our results agree roughly with those of other investigators.9–13 In each of three shallow water species, Mustelus manazo, Negaprion brevirostris and Ginglymostoma cirratum, the length of rod outer segments varied between 15 μm and 17 μm, with the average of 16 μm.9–11 Using 6 species collected from different pelagic environments, Gruber et al.12 made detailed measurements on the size and number of photoreceptors in paraffin sections. They found that the mean length of outer segments rod of the six species was 21.9 μm, with a range of 17.3 to 29.2 μm. With regards to deep sea sharks, Denton and Nicol13 found for the rods a mean length of 45 μm with a range from 43 to 48 μm.

The presence of cone has been described in several elasmobranch families (Orectolobidae, Alopiidae, Lamnidae, Carcharhinidae, Sphyridae, Squalidae, Rhinobatidae, Torpedinidae, Dasyatidae and Paratrygonidae).10–11,14–18 The results in the present study agree with the reports on the presence of cones in coastal and pelagic species, and on notable feature of their variability in shape. One may speculate, as did Walls,5 that this ambiguous diversity represents an evolutionary transition between rods and cones. The main difference in the cones of various species, other than in size and number, was in the location of their nuclei. Most nuclei of Dasyatis were situated on the receptor side of the limiting membrane and easily identified, while those of sharks were located deep in the outer nuclear layer and difficult to differentiate from those of the rods.

Walls5 observed that the outer nuclear layer was much thicker than the inner nuclear layer in nocturnal animals while the relationship was reversed in diurnal species. It is interesting to compare the thickness of the outer nuclear and outer plexiform layer (tONL) with the thickness of the inner nuclear layer (tINL) in the fishes examined in this investigation (Table 2). The ratio (tONL/tINL) is 0.32–0.75 in P. glauca, T. scyllia, D. akajei, D. kuhlii, U. auranticus and P. sinensis, while it is 1.45–2.98 in A. macrorhynchos, E. lucifer, D. eglantina, S. obscurus and C. tessellatus. The ratio was less than 1 in the fishes living in shallow waters or having pelagic behavior, whereas in deep sea fishes the ratio was generally observed to be more than 1.

The discussions done in this paper lead us to conclude that deep sea elasmobranchs, in short, show retinal adaptations enabling them to make use of the very dim light to which they are exposed. However, the retinal structure of elasmobranchs is rather standardized as shown in our figures, and the dramatic variations of retinal structures which often occur in teleosts are not observed in elasmobranchs. With respect to this matter, it is interesting that the teleosts devoid of reflecting tapeta have rods that are about twice as long and a retinal photopigment density that is about twice as great as in elasmobranchs, which have evolved an efficient redundant tapetum.13 It is speculated that this could be of advantages to the elasmobranch in that it could lead to an improved signal-to-noise ratio, hence to a faster dark adaptation without sacrifice of sensitivity. We considered that the increment of rod outer segment length in deep sea elasmobranchs was beneath notice when comparing it with that of teleosts. In conclusion, distinctive adaptation in elasmobranchs and teleosts seems to be seen in adaptive strategies to light environments.

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