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Temporal and mosaic distribution of large ganglion cells in the retina of a daggertooth aulopiform deep –sea fish (*Anotopterus pharao*)

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Temporal and mosaic distribution of large ganglion cells in the retina of a daggertooth aulopiform deep-sea fish (*Anotopterus pharao*)

M. Uemura[1], H. Somiya[1*], M. Moku[2] and K. Kawaguchi[2]

[1]*Faculty of Bioresources, Mie University, 1515 Kamihama, Tsu, Mie 514, Japan*
[2]*Ocean Research Institute, University of Tokyo, 1-15-1 Minamidai, Nakano, Tokyo 164, Japan*

The daggertooth *Anotopterus pharao* (Aulopiformes: Anotopteridae) is a large, piscivorous predator that lives within the epipelagic zone at night. In this species, the distribution of retinal ganglion cells has been examined. An isodensity contour map of ganglion cells shows that the cells concentrate in a slightly ventral region of the temporal retina. The region of high ganglion cell density contains 4.07×10^3 cells mm^{-2}, and the resulting visual acuity is 3.5 cycles deg^{-1}. Outside the area centralis, conspicuously large ganglion cells (LGCs) are observed in the temporal margin of the retina. The LGCs are regularly arrayed, and displaced into the inner plexiform layer. Thick dendrites extend into the outer part (sublamina a) of the inner plexiform layer. In the retinal whole mount, the total number of LGCs is 1590 (90.7 cm specimen), and the mean size of the LGCs is about four times larger than that of the ordinary ganglion cells. The morphological appearance of the LGCs was similar to the off-type alpha cells of the cat retina. The function of these distinctive LGCs is discussed in relation to specific head-up feeding behaviour.

Keywords: daggertooth; off-type large ganglion cell; temporal; mosaic distribution; feeding behaviour

1. INTRODUCTION

The daggertooth *Anotopterus pharao* (Aulopiformes: Anotopteridae) is a large, piscivorous predator that lives in the epipelagic zone at night, but is usually found below 1000 m during daytime (Heemstra 1990). The fish is the sole member of the daggertooth family. They are widespread throughout the world's oceans and are found in temperate and subarctic regions but not in tropical waters. Their body is extremely slender and scales and dorsal fin are absent. The teeth are remarkably sharp and dagger like as its name implies. In the north-west Pacific Ocean, the principal prey of the daggertooth is immature salmon of the genus *Oncorhynchus* (Balanov & Radchenko 1998). It is also suggested that the daggertooth may wait for prey with a head-up position, as is characteristic of many mesopelagic predators.

In the course of histological studies of deep-sea fishes' eyes, we unexpectedly found extremely large ganglion cells (LGCs) that were distributed only in the temporal retina of the daggertooth. In the present study, we have examined the morphological features and number of the LGCs of the fishes. The LGCs were laid down in regular mosaics in the temporal margin of the area centralis (AC). The total number of the LGCs was 1590 in the large specimen (standard length, 90.7 cm). The function of the LGCs is discussed in relation to the head-up feeding behaviour.

2. MATERIAL AND METHODS

Three different-sized specimens of the daggertooth preserved in 10% formalin were used for the present study. The standard lengths of the small, medium-sized, and large specimens were 52.8, 63.5 and 90.7 cm, respectively. These specimens were sampled from the north-west Pacific Ocean by nets of deep-sea trawlers at about 500–700 m depth on 24–30 July 1996. The trawling location was 39° 0′ to 41° 4′ north (latitude) and 143° 3′ to 144° 3′ east (longitude). The large and medium-sized specimens were caught in the daytime (500–600 m) and the small one at night (600–700 m). The retinas of right eyes from the medium and small-sized specimens were embedded in paraffin, cut into 7–10 μm serial radial sections, and stained with haematoxylin–eosin (H–E) or occasionally with the Bodian–Otsuka method (Otsuka *et al.* 1960). A part of the temporal retina from the large specimen was embedded in Epon Araldite, cut into 0.5–1 μm radial sections, and stained with toluidine blue. The remaining part of the retina from the large specimen and the left retina of the medium-sized specimen were embedded in paraffin, sectioned tangentially and radially, and stained with the H–E method or Bodian–Otsuka method.

In two specimens (90.7 and 52.8 cm), using the left eye, retinal whole-mount preparations were made following a protocol of Ito & Murakami (1984). These whole mounts were mounted on 3% gelatinized slide, and stained with cresyl violet (0.025%) for counting ganglion cells. The ganglion cells were counted according to the method of Collin & Pettigrew (1988). All recognizable neural elements lying within the ganglion cell layer were counted. Thus the densities calculated represent upper limits. In the large specimen, all regions of the retina were examined at regular intervals of 1 mm at ×400 magnification. In this way, 204 areas were sampled and an isodensity contour map drawn. We estimated the peak retinal resolving power according to a previous protocol (Collin & Pettigrew 1989).

For measurements of cell soma areas from the whole-mount preparation of the large specimen, 100 cells within the ganglion

*Author for correspondence (somiya@bio.mie-u.ac.jp).

cell layer were sampled in each designated area (each peripheral region of nasal, dorsal, temporal, ventral areas and the AC). Outlines of somata were drawn with a camera lucida and then imported into the program NIH Image (http://rsb.info.nih.gov/nih-image/) on a computer using a scanner. These methods are basically the same as those of Collin *et al.* (1998). Retinal shrinkage was not corrected for in the ganglion cell size measurements and contour map.

3. RESULTS

(a) *Eye, retina and photoreceptors*

Eyes of the daggertooth are almost circular and have a triangular hollow on the nasal side to gain a wider visual field (figure 1*a*). The lens muscle is thread like, suggesting that visual accommodation by lens displacement is difficult (Somiya 1987).

The retina was composed of ten-layer elements (figure 1*b*) as seen in shallow-water fishes. The thickness of the ventro-temporal quarter of the retina was about 250–290 μm (figure 1*b*(i)), and the rest of the retina was 210–250 μm (figure 1*b*(ii)). In the visual cell layer, two types of photoreceptors were distinguished, a single cone-like and a rod-like photoreceptor (figure 1*c*,*d*). Twin cones were not observed throughout the retina. Although the daggertooth lives in the deep sea, there were not many rods (rod-like cells) compared with retinas of other deep-sea fishes. By contrast, almost pure cone areas were observed in the temporoperipheral retina, where we found LGCs (figure 1*d*).

(b) *Ganglion cell distribution*

Two kinds of ganglion cells were observed, one was ordinary sized and the other was conspicuously large (figure 1*e*). LGCs were observed only in the temporal retina, where the LGCs existed in the inner plexiform layer (figure 1*e*). First, we describe the distribution pattern of the ordinary ganglion cells including their visual axis and visual acuity, then a distribution pattern of the LGCs and their morphological properties will be presented.

(i) *Distribution pattern of ordinary ganglion cells*

Distribution pattern of the ganglion cells was not uniform. An isodensity map is shown in figure 1*f*. A well-defined AC was observed in the retinal whole mount of the daggertooth (figure 1*f*). The position of the highest density area was located in a slightly ventral part of the temporal retina. The peak density of the ganglion cells was $4.07 \times 10^3\,\mathrm{mm}^{-2}$, and the total number was presumed to be 264×10^3. A horizontal visual streak was not observed in this species. The position of the AC clearly indicates that the visual axis of the daggertooth is slightly upper-forward.

Visual acuity was calculated using the Matthiessen's ratio. The ratio states that posterior nodal distance (PND) is 2.55 times the radius of the lens. PND $= 2.55 \times 2.45\,\mathrm{mm}$ (radius) $= 6.25\,\mathrm{mm}$.

The angle (θ) subtending 1 mm on the retina is calculated by $\tan\theta = 1\,\mathrm{mm}/\mathrm{PND}\ (6.25\,\mathrm{mm})$; $\theta = 9.09°$.

Within the AC there are 4.07×10^3 cells mm^{-2} or 63.8 cells mm^{-1}.

The spatial resolution may then be calculated by obtaining the number of cells subtended by one degree of visual arc: cells degree^{-1} = density at AC/θ = 63.8 cells/9.09 degrees = 7.02.

Since at least two ganglion cells are needed to distinguish the light and dark boundaries from one cycle of a grating of the highest resolvable frequency, visual acuity (cycles deg^{-1}) = cells $\mathrm{degree}^{-1} \times 1/2 = 7.02/2 = 3.5$.

(ii) *Distribution pattern of LGCs*

There was a specialized region where LGCs were regularly arrayed (figure 1*e* and figure 2*a*,*b*). The region was restricted only to a narrow peripheral area of the temporal retina (figure 2*c*). The LGCs were clearly observed in both preparations of radial sections and retinal whole mounts (figure 1*e* and figure 2*a*,*b*).

The LGCs found in the temporal region had various distinctive features. All the LGCs had a thick axon in the vitreous side of the soma in the silver-impregnated preparations (figure 2*d*,*e*). Indeed, in the temporal retinal area, which contained the LGCs, numerous thick optic nerve fibres were observed (figure 1*e*). On the other hand, no such thick fibres were observed in other retinal areas (figure 1*b*). These observations evidently show that the large cells are all ganglion cells but not displaced amacrine cells. The LGCs are regarded as alpha-like ganglion cells due to their large soma size, the regular array of the mosaic (figure 2*b*) and their thick dendrites (figure 2*e*). Further, their dendritic branches enter 'sublamina a' of the inner plexiform layer (figure 2*e*), which indicates that the LGC is physiologically off-type.

In the retinal whole mount of the large specimen, the LGCs distribution area was 4% (8.52 mm^2) of the total retinal area (216.0 mm^2). Total number of LGCs was dependent on the specimen size. The total number of LGCs was 400 in the small specimen, but 1590 LGCs were counted in the large specimen. In the large specimen, the mean soma size of the LGCs calculated from the retinal whole-mount preparation was $401.7 \pm 107.8\,\mu\mathrm{m}^{-2}$ (mean ± s.d.; $n = 100$). On the other hand, the mean size of ordinary ganglion cells in the same region was $99.0 \pm 19.3\,\mu\mathrm{m}^{-2}$ ($n = 100$). Further, the mean soma sizes of the ordinary ganglion cells found in the nasal region, dorsal region, ventral region and AC were 131.7 ± 38.4, 125.5 ± 31.3, 102.4 ± 26.0 and 72.1 ± 12.3 $\mu\mathrm{m}^{-2}$ ($n = 100$), respectively.

The shapes of the LGCs resembled each other in the retinal whole-mount preparations. In radially sectioned preparations, however, shapes of the cells were various, such as round, cone shaped and pear shaped (figure 1*e* and figure 2*d*). In addition, their positions of somata were not in the ganglion cell layer but were displaced into the inner plexiform layer. Two to four thick primary dendrites extended to the outer part of the inner plexiform layer that is called 'sublamina a' (figure 2*e*). In contrast, somata of LGCs in the small specimen were mostly cone shaped, and were not displaced into the inner plexiform layer.

The LGCs were regularly arrayed (figure 2*b*). In the peak density area of the LGCs (525 cells mm^{-2}), a calculated nearest-neighbour distance was 42.2 ± 5.1 μm ($n = 20$). This regular mosaic distribution indicates that it is a single cell type, and that they are all alpha-like off-type ganglion cells. The visual axis for the LGCs was

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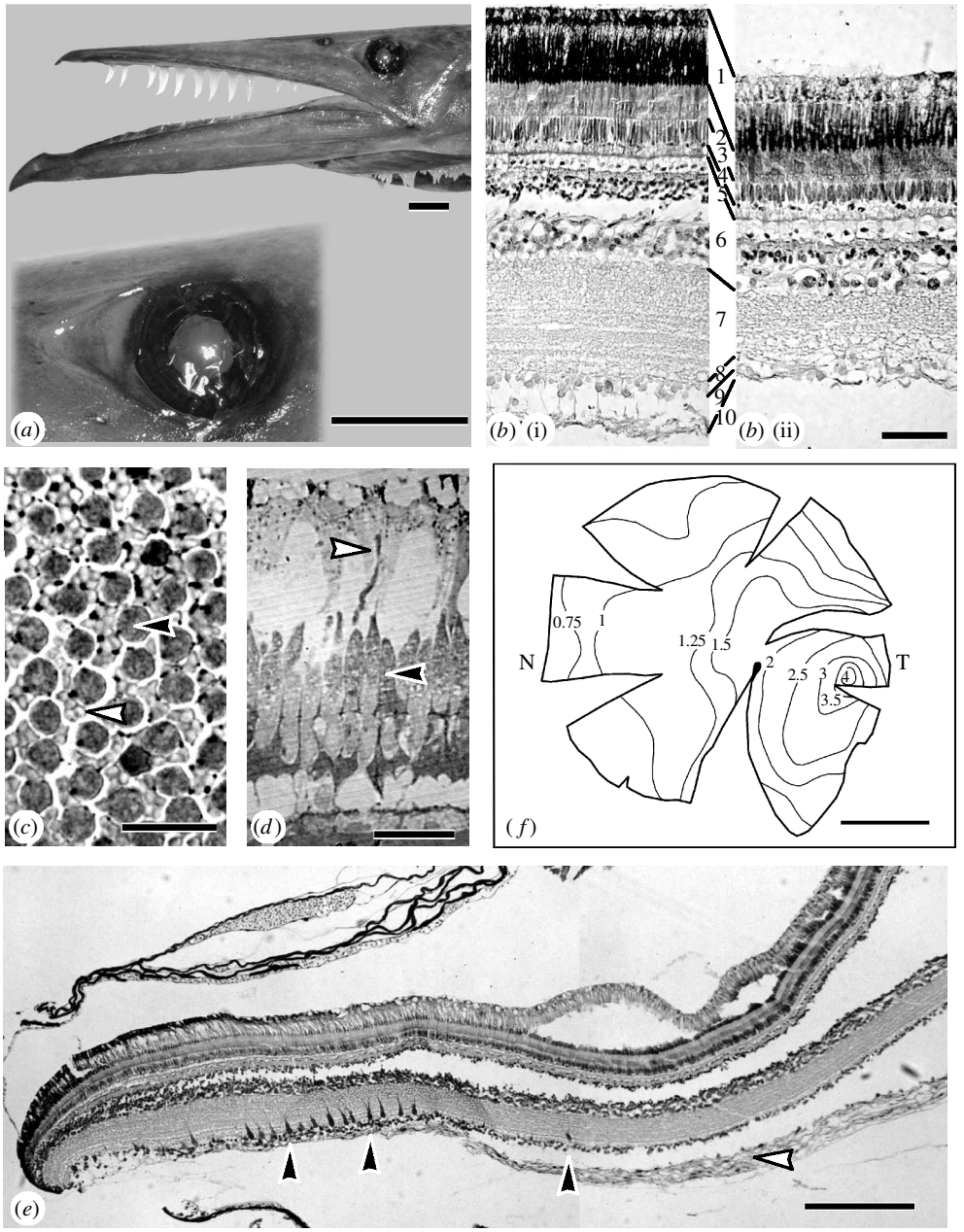

Figure 1. (*a*) Head and close up of eye of *A. pharao* showing its dagger-like teeth on the upper jaw and its triangular hollow on the nasal side. Scale bar, 1.5 cm. (*b*) Radial section of the AC (*b*(i)) and the central fundus of the retina (*b*(ii)) showing its ten-layer elements stained with the Bodian–Otsuka method. 1, pigment epithelium layer; 2, visual cell layer; 3, external limiting membrane; 4, outer nuclear layer; 5, outer plexiform layer; 6, inner nuclear layer; 7, inner plexiform layer; 8, ganglion cell layer; 9, nerve fibre layer; 10, internal limiting membrane. Scale bar, 50 µm. (*c*) Tangential section of the central part of the retina showing its two types of photoreceptors stained with H–E. The black arrowhead indicates the cone-like outer segment. The white arrowhead shows the rod-like outer segment. Scale bar, 20 µm. (*d*) Radial section of the temporoperipheral retina showing the almost pure-cone region, stained with toluidine blue. The black arrowhead shows a single cone. Rods (white arrowhead) are uncommon in the temporal region. Scale bar, 20 µm. (*e*) Radial section of the temporal retina, stained with H–E. The black arrowheads indicate LGCs discovered only in the temporoperipheral region. The white arrowhead shows numerous thick optic nerve fibres. Scale bar, 200 µm. (*f*) Isodensity contour map of the distribution of the ordinary retinal ganglion cells of the large specimen. T, temporal; N, nasal. Scale bar, 5 mm.

estimated to be directing forward. The visual acuity for the LGCs vision was calculated as 1.2 cycles deg^{-1}.

4. DISCUSSION

A group of LGCs was observed only in the temporal margin of the retina in the daggertooth (figure 1*e* and figure 2*a*). The LGCs were ectopic and displaced into the inner plexiform layer (figure 2*d*,*e*). In the large specimen, the peak density of the LGCs was 525 cells mm^{-2}, with a total of 1590 cells counted. The mean soma area (*ca.* 400 µm^2) of the LGCs was four times larger than that (*ca.* 100 µm^2) of the ordinary-sized ganglion cells in the same temporal retina. The LGCs were pear shaped and arranged in a regular mosaic (figure 2*b*). Further, all of their dendrites entered 'sublamina a' of the inner

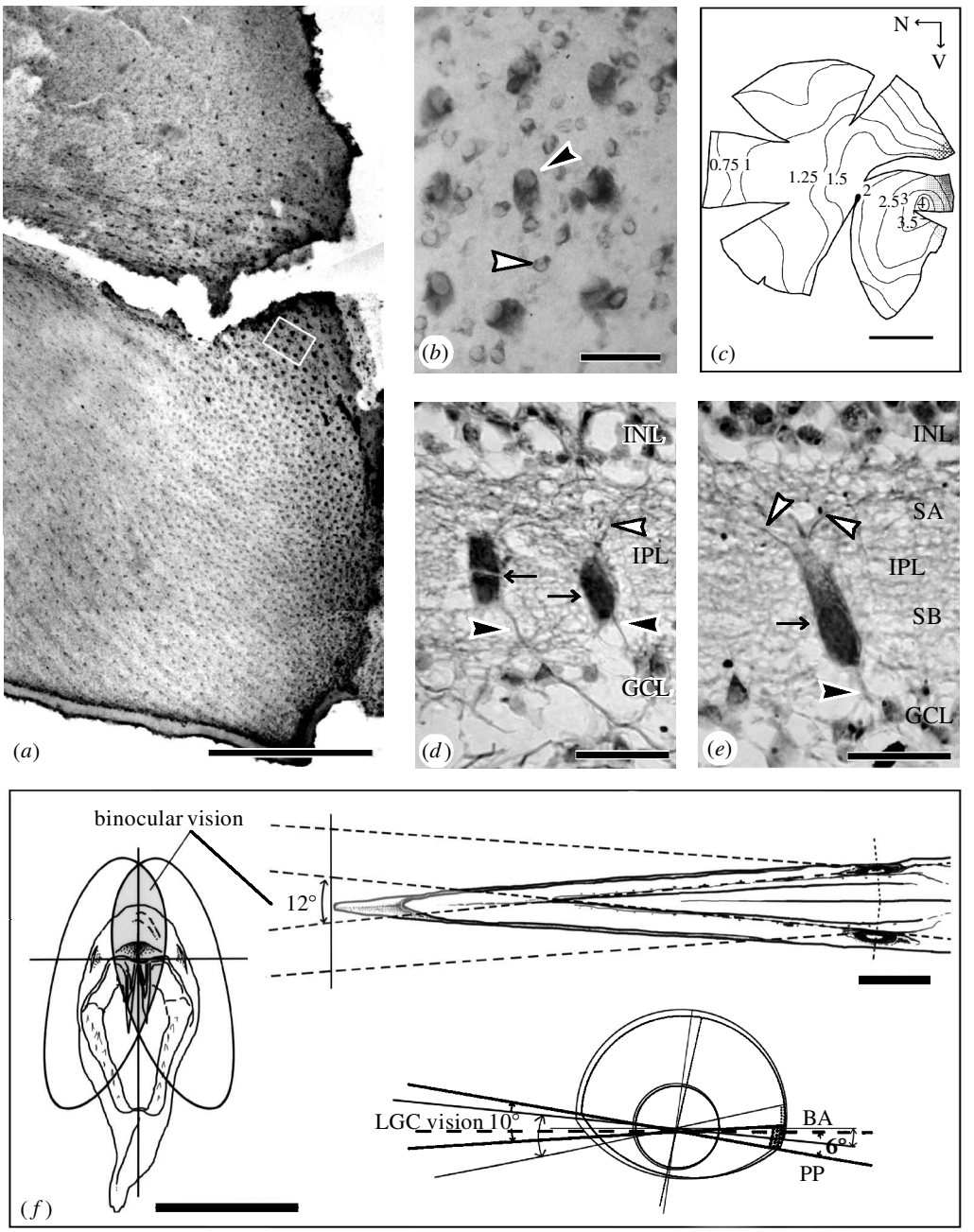

Figure 2. Regularly arrayed LGCs of the temporoperipheral region of the retinal whole mount of the large specimen, stained with cresyl violet. The small rectangle encloses a region shown in (*b*). Scale bar, 1 mm. (*b*) The black arrowhead indicates the LGC. An ordinary ganglion cell is shown by a white arrowhead. Scale bar, 50 μm. (*c*) The stippled area in the temporal region indicates the distribution area of LGCs. N, nasal; V, ventral. Scale bar, 5 mm. (*d*,*e*) Radial sections of the LGCs of the temporoperipheral retina stained with the Bodian–Otsuka method. Arrows indicate their somata displaced into the inner plexiform layer. Black arrowheads indicate their thick axons. The dendrites (white arrowheads) extend to 'sublamina a' of the inner plexiform layer. INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; SA, sublamina a; SB, sublamina b. Scale bar, 30 μm. (*f*) Dorsal and frontal view of the head showing the binocular visual field based on the distribution area of off-alpha-like LGCs. Enlarged left schematic eye in meridianal section and its LGC vision are also shown. Where the radius of the lens is 2.45 mm the focal length (PND) is 6.25 mm. The measured horizontal width of the LGC area is *ca*. 2 mm. The angle (α) subtending 2 mm on the retina can be calculated by $\tan\alpha = 2\text{ mm}/\text{PND} = 2\text{ mm}/6.25\text{ mm}$; $\alpha = ca.$ 18°. BA, body axis; PP, pupillary plane of the eye. Scale bar, 1 cm.

plexiform layer (figure 2*e*). Therefore, we consider that they are physiologically 'off-centre Y-like' (Famiglietti *et al*. 1977) and morphologically 'alpha-like' cells as reported in the cat retina (Wassle *et al*. 1981).

Similar 'off-alpha-like' cells have been described in the retina of the cichlid *Oreochromis spilurus* (Cook & Becker 1991). However, their study showed the off-alpha-like cell population to be distributed across the entire retina, in contrast to the restricted distributions in the daggertooth.

The visual field of the LGCs is shown in figure 2*f*. Binocular vision with an angle of 12° was observed in a forward direction at the tip of the beak of the fish in the

horizontal axis and with an angle of 30° in the lateral plane. In contrast, the visual field with a high resolving power of the AC was monocular without eye movement and directed upper-forward at 20° to the body axis.

A similar area has been described in the temporal retinas of five procellariiform sea birds (Hayes *et al.* 1991). The peak density of the alpha-like ganglion cells in the sea bird *Puffinus puffinus* was 100 cells mm^{-2}, and the mean soma area (640 μm^2) of the LGCs was 3.2 times larger than that of ordinary-sized ganglion cells (200 μm^2) in the same temporal retina. (These values were calculated from fig. 3 in Hayes *et al.* (1991).) Although, the cells of the sea bird were much bigger than those of the daggertooth, the ratio between soma areas of LGCs and ordinary ganglion cells is nearly the same as in the present observation. One intriguing difference between these similar LGCs of the sea bird and the daggertooth is that the cells of the sea bird were physiologically 'on-type', but are considered to be 'off-type' in the daggertooth. This interesting point will be discussed later. Hayes *et al.* (1991) named the area as 'area giganto cellularis' and suggested the function as 'in the detection of objects on the sea surface from above'.

Recently, Collin *et al.* (1998) found the same retinal area giganto cellularis in the tubular eye of the deep-sea pearleye (*Scopelarchus michaelsarsi* and *Scopelarchus analis*: Scopelarchidae). The pearleye and the daggertooth belong to the same order of Aulopiformes. In their large specimen (61 cm in standard length), parameters of the area giganto cellularis were as follows: (i) the peak density of the LGCs was 791 mm^{-2}, (ii) a total number of LGCs was 520, (iii) per cent of area occupied by LGCs to the total retinal area was 3.2%, (iv) mean LGC soma size to mean ordinary ganglion cell soma size was about four times, and (v) upper limit of visual acuity of LGC was 0.9 cycles deg^{-1} (Collin *et al.* 1998). Except for the soma size of ganglion cells (soma size of LGCs and ordinary ganglion cells in the daggertooth was ten times bigger than those of the cells in the pearleye), the rest of the parameters of the LGC were nearly the same in both deep-sea fishes. Collin *et al.* (1998) suggested that the alpha-like LGCs may be motion sensitive.

Recent advances in the fisheries biology of salmon in north Pacific waters revealed that a principal predator for immature and even adult salmon is the daggertooth (Welch *et al.* 1991; Radchenko & Semenchenko 1996; Balanov & Radchenko 1998). It has also been shown that the daggertooth is mainly responsible for 'incised' slashes, which run at an angle of *ca.* 45° on only one side of the salmon body, because the daggertooth attacks the salmon from below (Balanov & Radchenko 1998). From these results, Balanov & Radchenko (1998) assumed that the daggertooth lies with its head up in anticipation of its prey as do many mesopelagic predators, such as paralepid (Janssen *et al.* 1992).

In considering this recent information regarding the daggertooth biology, we can speculate on their visual ecology for prey hunting. In the epipelagic zone, the daggertooth will hover head up with the body at about 70°. In this position, the LGC vision is along the body axis, while the high resolving vision of the AC is directed just upward to scan wider ranges of the field. In such lie (hide)-in-wait hunting behaviour with a head-up position, the daggertooth may use its eyes predominantly where it may hover using only the caudal part of the trunk muscle to minimize self-induced oscillations and stabilize the eye position, as do the paralepids (Janssen *et al.* 1992). As soon as the LGCs detect any black silhouette crossing the retina from right to left or vice versa in their LGC visual field, the fish lunges towards it. It is well known that the off-type Y cell responds transiently to the onset of a dark spot in the cat retina (Saito *et al.* 1970). It is reasonable that the daggertooth adopts the off-cell for this specific retinal area, because the fish attacks its prey from below. From this point of view, it is also realistic that sea birds use the on-cell, because the birds detect the prey from above. If the pearleye looks for bioluminescent point sources in the deep-sea environment, they may adopt the on-type.

Finally, the area giganto cellularis was observed in the temporal margin of the retina in the deep-sea daggertooth as well as sea birds (Hayes *et al.* 1991) and deep-sea pearleyes (Collin *et al.* 1998). The regular mosaic distribution of the LGCs in a peripheral zone of the temporal retina suggests that it may be related to the structural basis of binocular vision in some aspects. If this speculation is true, many more animals may possess an area giganto cellularis in the retina. This point and the central projections of the LGCs will be the subject of future research.

We would like to dedicate this short paper to Sir Eric Denton for his pioneering works on deep-sea fish vision. We thank Professor Hironobu Ito of Nippon Medical School and Dr Quentin Bone FRS of the Plymouth Laboratory for reviewing the manuscript. Part of the study was carried out at the Fisheries Research Laboratory (Wagu) and the Training Ship *Seisui Maru*; we thank Dr K. Kimura and Director I. Ishikura and other staff for their kind hospitality.

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