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Retinal specialisations in the dogfish *Centroscymnus coelolepis* from the Mediterranean deep-sea*

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SUMMARY: The present work attempted to study the importance of vision in *Centroscymnus coelolepis*, the most abundant shark in the Mediterranean beyond a depth of 1000 m, by using anatomical and histological data. *C. coelolepis* exhibited large lateral eyes with a large pupil, spherical lens and a *tapetum lucidum* that gave the eye a strong greenish-golden "eye shine". In the outer retinal layer, a uniform population of rod-like photoreceptors was observed while in the vitreal retina a thick inner plexiform layer comprised up to 30% of the whole retinal thickness. The cell distribution of the ganglion cell layer formed a thin elongated visual streak in the central plane of the eye that provided a horizontal panoramic field of view. A specialised area of higher visual acuity was located caudally at 32-44º from the geometric centre of the retina and 5-10º above the horizontal plane of the eye. This position indicated that the visual axis pointed in a slightly outward-forward direction with respect to the fish body axis. A non-uniform distribution of large ganglion cells was also found in the horizontal plane of the retina that practically coincided with the distribution of the total cell population in the ganglion cell layer. This is the first time that this type of retinal specialisation has been observed in the elasmobranchs. These characteristics indicate that the retina of *C. coelolepis* is designed not only to increase sensitivity in the horizontal field of view, as was also observed in other sharks, but also to improve motion detection in the same plane. The visual capacities evolved by *C. coelolepis* make this species adapted for discriminating the horizontal gradation of light that exists in the mesopelagic environment. Similarly, the large ganglion cell distribution observed in its retina seems to be related to its predatory behaviour, since it allows this shark to perceive the movement of bioluminescent prey against a totally dark background.

Key words: deep-sea shark, retinal morphology, retinal topography, large ganglion cells, visual axes, diet.

RESUMEN: ESPECIALIZACIONES EN LA RETINA DEL TIBURÓN *CENTROSCYMNUS COELOLEPIS* DEL MEDITERRÁNEO PROFUNDO. – El presente trabajo estudia la importancia de la visión en *Centroscymnus coelolepis*, el tiburón más abundante en el Mediterráneo por debajo de los 1000 m de profundidad, usando datos anatómicos e histológicos. *C. coelolepis* posee grandes ojos laterales, una pupila muy ancha, un cristalino esférico y un *tapetum lucidum* que confiere al ojo un fuerte brillo verde-dorado. En la capa externa de la retina, se observa una población uniforme de fotorreceptores parecidos a los bastones, mientras en la capa interna el estrato plexiforme interno es grueso, ocupando hasta el 30% del entero grosor de la retina. La distribución de las células en la capa ganglionar forma una sutil franja horizontal alargada en correspondencia del plano central del ojo que permite al animal una visión panorámica horizontal. Caudalmente se localizó una área especializada de mejor agudeza visual a 32-44º desde el centro geométrico de la retina y 5-10º por encima del plano horizontal del ojo. Esta posición indica que el eje visual está dirigido hacia afuera y hacia adelante respecto el eje mediano del cuerpo. En el plano horizontal de la retina se observó también una distribución no uniforme de células ganglionares grandes que prácticamente coinciden con la distribución de todas las células en la capa ganglionar. Esta es la primera vez que se observa este tipo de especialización en la retina de un elasmobranquio. Todas estas características indican que la retina de *C. coelolepis* está diseñada no solo para incrementar la sensibilidad visual en el plano horizontal, como se ha observado también en otros tiburones, sino también para aumentar la detección del movimiento en el mismo plano. Las capacidades visuales con que ha evolucionado *C. coelolepis* hacen que esta especie esté adaptada para discriminar la gradación horizontal de luz que existe en el medio mesopelágico. Así mismo, la distribución de las células ganglionares grandes observadas en su retina parece estar relacionada con su comportamiento predador permitiendo al tiburón percibir el movimiento de sus presas bioluminescentes contra un fondo totalmente oscuro.

Palabras clave: tiburón de profundidad, morfología retiniana, topografía retiniana, células ganglionares grandes, ejes visuales, dieta.

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INTRODUCTION

Vision underwater is severely limited by the optical properties of the water. In the Mediterranean Sea, at most 1% of the surface light reaches a depth of 150 m and only 0.1% reaches a depth of 225 m (Ivanoff, 1975). Deeper than 200 m, light intensity declines less rapidly. Clarke and Denton (1962) considered that, in optimal conditions of very clear water, fishes should be able to distinguish daylight down to about 1000 m. On the other hand, depending on the physical conditions of the water, the greatest depth for vision could vary between 700 and 1300 m, where the homochromatic blue light is centred at around 480 nm. Below these depths, there is an "optically empty" environment (Locket, 1977). In the Mediterranean, the maximum depth for fish vision has not been calculated for any fish. However, a light intensity of $2 \cdot 10^{-7}$ μE m^{-2} s^{-1} (Aguzzi, 2002) found at a depth of 400 m in summer is similar to the minimum illumination ($5 \cdot 10^{-7}$ μE m^{-2} s^{-1}) required to activate a visual foraging response in the walleye pollock *Theragra chalcogramma* (Ryer and Olla, 1999), which can live at depths of more than 900 m.

These findings indicate that light is of interest to the animals that live in a deep-sea environment. Moreover, the eyes of meso- and bathypelagic fish show particular adaptations to dim light environments that allow them to increase visual sensitivity and detect blue light (Douglas and Partridge, 1997), although this light does not come from the sun but from other animals in the form of bioluminescence. In fact, bioluminescence is common in the ocean, from the surface to the deepest trenches, and the ability to make light in a dark world has such a pronounced advantage that it has evolved perhaps as many as thirty times (Hastings, 1983).

The Portuguese dogfish, *Centroscymnus coelolepis* Bocage and Capello, 1864, dwells in the deep-sea between the mesopelagic and the bathypelagic zones, where vision is strongly limited. This species is widely distributed around the world and its depth distribution ranges from 270 to 3700 m (Compagno, 1984). During the DESEAS cruise in the Mediterranean (financed by the EC), it was caught at depths of 1500, 2500 and 2800 m (cf. Sion *et al.*, 2004). As sharks occupy a high level in the marine trophic chain, identifying the special role of their sensory organs is one of the means of understanding their behavioural response. Although vision in sharks has always been considered as a "secondary" sensory organ in comparison with the chemosensory, mechanoreceptive and electroreceptive organs, several studies recognise the significant role of vision in shark behaviour (see Hueter and Cohen, 1991 for a review). Sharks have well-developed eyes, whose retina is sometimes provided with rods and cones. Many of these fish have a *tapetum lucidum* behind the retina, a reflecting system that enhances photon capture of the retina and allows the animals to see in very dim light environments. Nevertheless, vision of deep-sea sharks is only partially understood. Denton and Shaw (1963) investigated the visual pigments of some deep-sea elasmobranchs and found that *C. coelolepis* showed appreciable absorption at 472 nm, close to the wavelength at which the sea is most transparent. In 1991, Crescitelli (1991) found that visual pigment in the brown cat shark *Apristurus brunneus*, trawled from a depth of 550 m, absorbed maximally at 483 nm. Bozzano and Collin (2000) also investigated visual specialisation in terms of retinal topography and visual acuity in a range of species occupying different habitats and depths, from the surface to a depth of 3000 m.

The analysis of vision in the Portuguese dogfish *Centroscymnus coelolepis* is particularly relevant because it is the most abundant shark at great depths in the north-western Mediterranean and its distribution is almost exclusively restricted to the lower slope below 1000 m, where sunlight is visually irrelevant. In addition, recent investigations postulate that Mediterranean individuals of *C. coelolepis* belong to a segregated population due to their lower size and preferential deeper distribution than Pacific and Atlantic populations (Cló *et al.*, 2002).

MATERIALS AND METHODS

Sample collection

The individuals of *Centroscymnus coelolepis* were collected in June 2001 during the DESEAS cruise (Sardà *et al.*, 2004), which sampled at 1400-1500 m using an OTMS bottom trawl (Sardà *et al.*, 1998). Fish total length (TL) was recorded to the nearest 0.5 cm. For the current analysis two adult individuals of 60 and 63.5 cm TL were chosen. From fresh individuals, the eyes were excised and the axial and equatorial eye diameters were measured to the nearest 0.1 cm. The cornea and lens were then cut away from the excised eyes and the axial and equatorial diameters of the unfixed lens were

measured. The eyecup was fixed in 4% paraformaldehyde, 2.5% gluteraldehyde and 3% sucrose in 0.1M phosphate buffer for 24 hours, then transferred to 0.1M phosphate buffer and stored at 4ºC. The spherical index of the eye and the lens was calculated as the quotient between the equatorial and axial diameter, in accordance with Branis (1981).

Structure of the retina

The right eyes were employed for the analysis of the retinal histology. Due to the large size of the eyes, these analyses were carried out only on the rostro-centro-caudal portion of the retina. The retinae were dehydrated in an ethanol series and embedded in Historesin (Leica). Radial semithin sections (1-3 μm) were cut with a Reichert-Jung microtome and stained with methylene blue and basic fuchsin. For the measurements of the thickness of the retinal layers, the sections with the exit of the optic nerve were selected. The measurements of the thickness of each retinal layer and photoreceptor density were taken 300 μm from the *ora serrata*. Rod density was calculated according to the Van der Meer and Anker (1986) equation:

$$\text{Density} = 10^6\, m\, [(t + d - 2f)\, w]^{-1} \text{ cells per mm}^2$$

where m is the mean number of cells counted, t is the section thickness, d is the mean diameter of the cells, f is the thickness of the smallest cell fragment counted (or $f = 0.1$ d) and w is the width of the sampled strip.

Retinal topography of cells in the ganglion cell layer

The left eyes were employed for the analysis of the retinal topography. The retinae were removed from the scleral eyecup and teased free of the underlying retinal pigment epithelium and the choroidal *tapetum lucidum*. Peripheral slits enabled the retinae to be whole-mounted according to Collin (1988). Each retina was then stained for 1 min in 0.05% cresyl violet, dehydrated, cleared, coverslipped with DPX, and the ganglion cell layer was examined for cells containing Nissl substance. Retinal shrinkage was not corrected.

The cells were routinely counted at 600x every 0.05 mm across the retina, but in higher density areas cell numbers were counted every 0.02 mm. In this way, approximately up to 1400 areas were sampled for each retina. These values were converted into cells per mm^2. Isodensity maps were constructed by interpolation between similar values of retinal ganglion cell densities, following the methods of Collin and Pettigrew (1988). All neural elements lying within the retinal ganglion cell layer were counted, excluding only cigar shaped and darkly stained cells considered to be glial cells (Hughes, 1985). A population of displaced amacrine cells known to be present in the ganglion cell layer of fish was included in the counting, since no retrograde labelling was attempted (Collin, 1988). The same type of counting was repeated only for those cells that had large soma and thick dendritic branches, and isodensity maps were constructed as described above. The retinal area and the measurement of the cell soma area in the ganglion cell layer were determined using an image analyser (Optimas 6.0).

The theoretical visual acuity was calculated for the maximum ganglion cell density according to the method of Collin and Pettigrew (1989), which calculated the minimum separable angle (MSA) subtended by the ganglion cells projected into visual space by a lens obeying Matthiessen's ratio (Matthiessen, 1880). The MSA angle is calculated as follows:

$$\text{MSA (min of arc)} = 60\, (2 \tan \alpha \sqrt{G^{-1}})$$

where α is the angle subtending 1 mm on the retina, which is in turn given by $\tan \alpha = 1$ mm/f. The focal length (f) is calculated by m·r, where r is the lens radius and m is the distance from the centre of the lens to the retina divided by the lens radius, which is assumed to be 2.55 for fish with a spherical lens (Matthiessen's ratio). G is the peak cell density located within the ganglion cell layer.

It should be noted that displaced amacrine cells were included in the counting, so the spatial resolving power was overestimated. Consequently, the values of resolving power obtained from the histological preparation give a predictable visual acuity that can be confirmed only through behavioural experiments.

The position of the visual axis was determined by measuring in mm the location (L) of the specialised areas on the isodensity map from the geometric centre of the retina. Then, the value in radians corresponding to this length was calculated as radians = L/f, where f is the focal length. This value in radians was converted into degrees. In this way, the linear measurement on the retina was converted into spherical coordinates, according to Mass and Supin

TABLE 1. – Summary of data on body size, ocular and retinal characteristics for two individuals of *Centroscymnus coelolepis*. GCL, ganglion cell layer; LGCs, large ganglion cells; MSA, minutes of arc; m, average in the entire retina; s.a., specialised area; p, periphery.

		Individual 1		Individual 2
Fish length (cm)		60		63.5
Depth caught (m)		1500		1400
Eye size (equatorial diameter / axial diameters) (mm)		26.1 / 25.0		27.5 / 25.5
Lens size (equatorial diameter / axial diameters) (mm)		11.7 / 11.5		12.3 / 11.5
Retinal surface (mm^2)		1275.1		1163.1
Total number of cells in the GCL		1181004.5		914067.43
Soma size of cells in the GCL (mean ± SD) (μm^2)	m	137.1 ± 22.1	m	151.9 ± 70.7
	s.a.	134.4 ± 52.8	s.a.	134.1 ± 61.4
	p	163.9 ± 71.1	p	169.8 ± 75.1
Soma size of cells (excluding LGC) in the GCL (μm^2)	m	83.3 ± 22.1	m	95.4 ± 23.8
	s.a.	79.9 ± 17.1	s.a.	81.8 ± 16.6
	p	86.4 ± 25.8	p	108.8 ± 22.3
Soma size of large ganglion cells in the GCL (μm^2)	m	190.8 ± 82.7	m	208.4 ± 55.2
	s.a.	149.5 ± 37.2	s.a.	186.2 ± 42.3
	p	240.8 ± 89.8	p	230.6 ± 57.9
Total number of large ganglion cells		112400.1		69827.5
Percentage of LGCs throughout the retina		9.5		7.6
Peak cell density in the GCL (cells mm^{-2})		3217		2950
Peak LGCs density (cells mm^{-2})		283		207
Peak density gradient		6.4:1		5.9:1
MSA (min of arc)		8.3		8.06
Caudal visual axis position (degrees)		32.3		40.8
Retinal thickness (mm)		80.2 ± 2.2		78.9 ± 3.7
Rod outer segment length (mm)		32.8 ± 1.3		30.2 ± 0.7
Inner plexiform layer length (mm)		22.6 ± 1.1		23.5 ± 2.0
Rod density ($n \cdot 10^3\ mm^{-2}$)	m	33.5 ± 3.1	m	34.1 ± 1.2
	s.a.	38.8 ± 2.5	s.a.	37.5 ± 4.1
	p	30.1 ± 1.1	p	30.7 ± 2.4

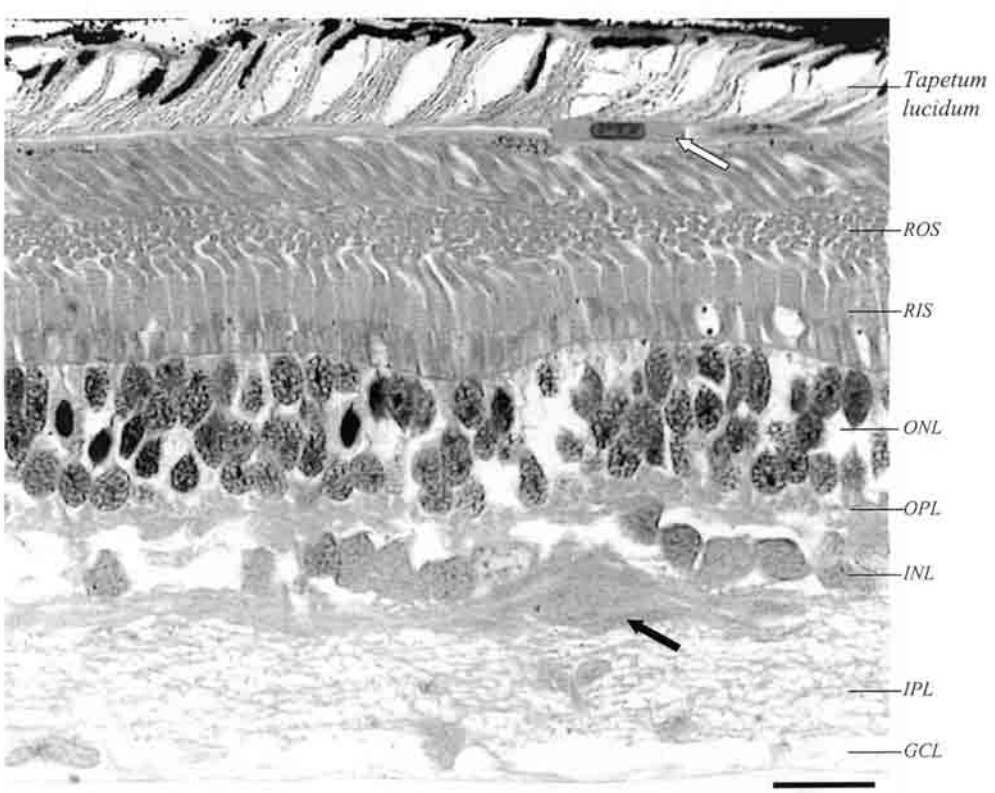

FIG. 1. – Light micrograph of transverse section of *Centroscymnus coelolepis* retina (individual of 60 cm TL). Only rod-like photoreceptors are present throughout the retina. Abbreviations: ROS, rod outer segment; RIS, rod inner segment; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer. White arrow indicates a red blood cell, black arrows indicate a displaced large ganglion cell. Scale bar 10 μm.

(1986) and Murayama *et al*. (1995). The vertical distance of the specialised area from the horizontal plane of the eye was measured as the cosine of the angle between the distance of the specialised area from the geometric centre of the retina, as measured on the map, and its vertical projection on the horizontal plane of the eye.

RESULTS

The eye and the retina

The eye spherical index was 0.96 in the individual of 60 cm and 0.93 in the individual of 63.5, which indicated that the eyes of large *Centroscymnus coelolepis* were roughly circular, as were their lenses (the lens spherical index was 0.98 and 0.93 for the two individuals respectively). During a previous analysis (unpublished data) of 20 *C. coelolepis* ranging between 20 and 65 cm TL, the eye and the lens spherical indices gave mean values of 0.93 ± 0.05 and 0.95 ± 0.04 respectively, also indicating that the eye and the lens in this species were almost spherical.

In fresh specimens, the eyes showed a strong greenish reflectance, indicating the presence of a *tapetum lucidum*. The optic nerve head was located at a centro-ventral position in the retina and the falciform process was not evident. The morphological measurements of the eye and the retina of *C. coelolepis* are shown in Table 1.

The thickness of the retina measured approximately 80 μm in the central region of the eye. In the outer retinal layer, a uniform population of rod-like photoreceptors, whose outer segments measured 30-32 μm, was observed (Fig. 1). The outer nuclear layer, composed of rod nuclei, had a thickness of up

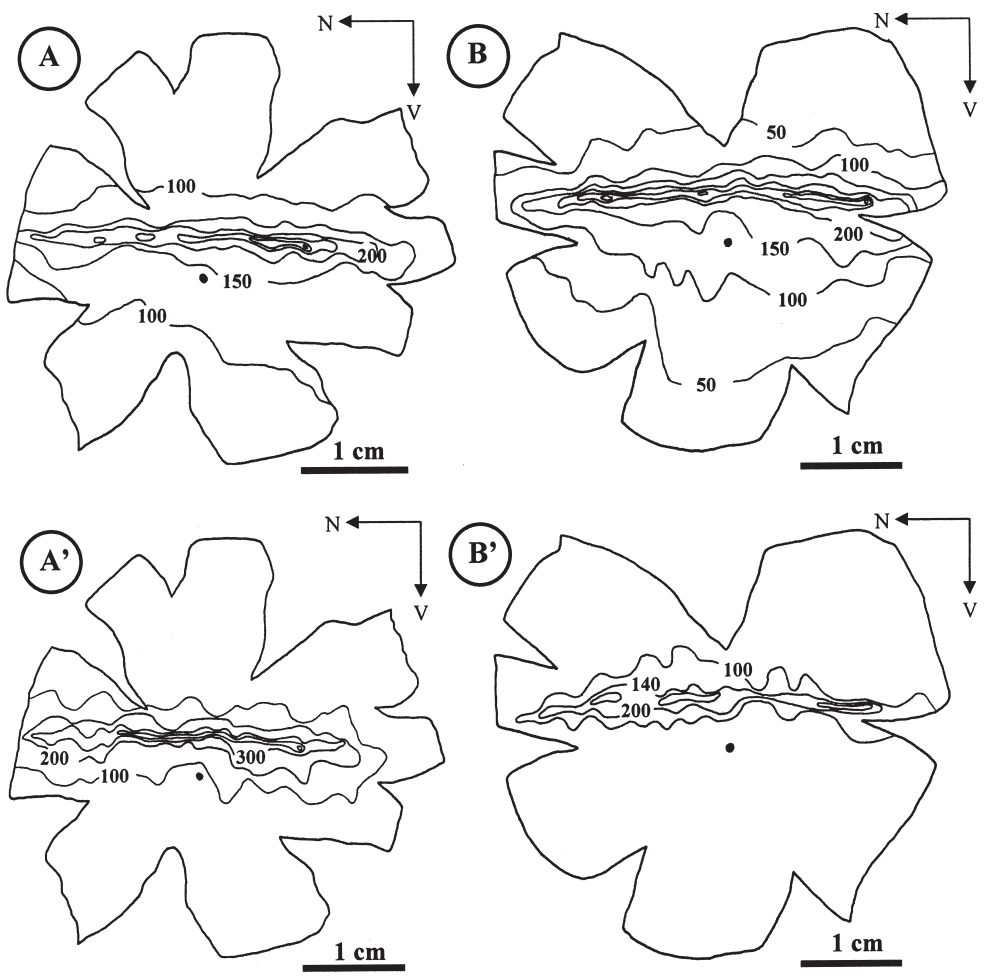

FIG. 2. – Retinal topography of the total cell population located in the ganglion cell layer (A and B) in the individuals of 60 and 63.5 cm TL respectively. Densities are x 10. A' and B', retinal topography of the large ganglion cells only. The optic nerve head is depicted in black. N, nasal; V, ventral.

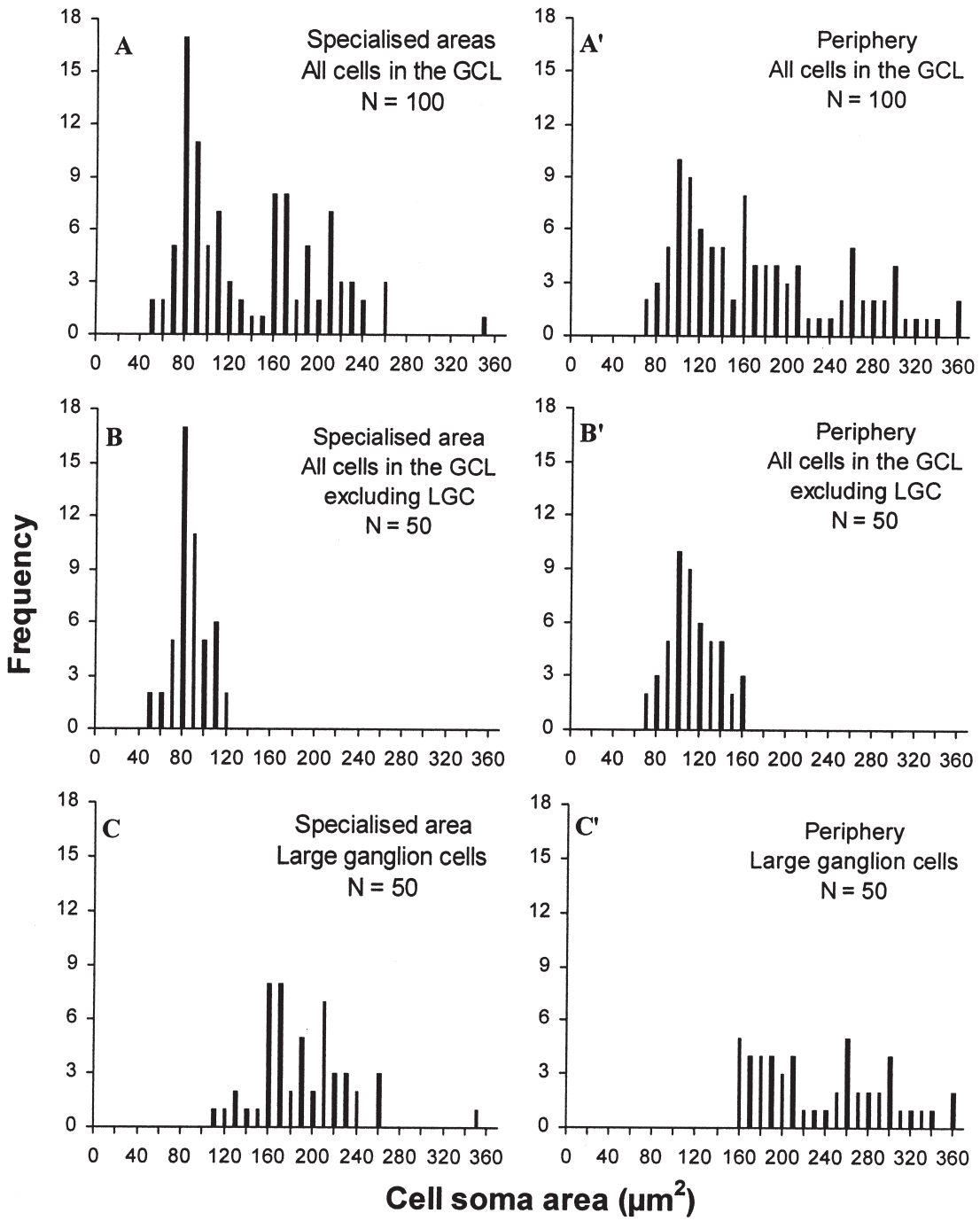

FIG. 3. – Frequency histograms of the total cell population soma area within the ganglion cell layer in the specialised area (A) and in the periphery (A'). B and B' are frequency histograms of soma area for the putative displaced amacrine cells and ordinary ganglion cells. C and C' are frequency histograms of the large ganglion cells. The mean and standard deviation are indicated in the text and in Table 1.

to 25 µm. The rod nuclei measured 4.6 ± 0.8 µm in diameter, and their density ranged between 30 x 10^3 cells mm^{-2} in the retinal periphery and 39 x 10^3 cells mm^{-2} in the central retina. The inner nuclear layer was poorly developed, while the inner plexiform layer was thick, constituting 25-30% of the whole retinal thickness, although this was mainly in the central portion of the retina.

In the vitreal retina, the ganglion cell density, calculated for the whole-mounted retina, was approximately 3000 cells mm^{-2} in the centro-caudal area and less than 500 cells mm^{-2} at the periphery, giving a density gradient of 6:1. The relationship between the ganglion cell density and the rod density in the specialised area indicated that approximately 12 rods converged on one single ganglion cell.

Retinal topography of cells in the ganglion cell layer

The cells in the ganglion cell layer were non-uniformly distributed throughout the retina. The isodensity distribution map (Fig. 2A, B) revealed a progressive increase of cell density from the dorsal and ventral retinal periphery to the central horizontal plane of the eye, forming an elongated visual streak. A specialised area of increased cell density was located in the centro-temporal retina, inside the horizontal streak. In this area, the theoretical visual acuity calculated for the maximum ganglion cell density was nearly 7 cycles per degree for both the analysed fish, which corresponded to 8 minutes of arc. However, this value could have been overestimated due to the inclusion of displaced amacrine cells in the count.

The cell soma size in the ganglion cell layer ranged between 50 and 360 μm^2 and the frequency distribution revealed more than one mode, indicating that more than one size class was present (Fig. 3 A and A'). The morphological appearance of the soma revealed different types of cells. Small, circular, darkly staining cells were presumed to be displaced amacrine cells, and medium-sized granular, irregularly shaped cells presumably represented the ordinary ganglion cells. Due to the difficulties in distinguishing between displaced amacrine cells with large soma and small ordinary ganglion cells, no difference was made in the measurement of the soma of these two types of cells that together ranged between 50 and 120 μm^2 (Fig. 3 B and B'). Cells with large less stained soma, up to three times the mean soma size of the ordinary cells, and thick dendritic branches (Fig. 4) ranged between 120 and 360 μm^2 (Fig. 3 C and C'). As no retrograde labelling of ganglion cells was attempted in this study, it was not possible to define these large cells as the giant ganglion cells found in other fish. The large ganglion

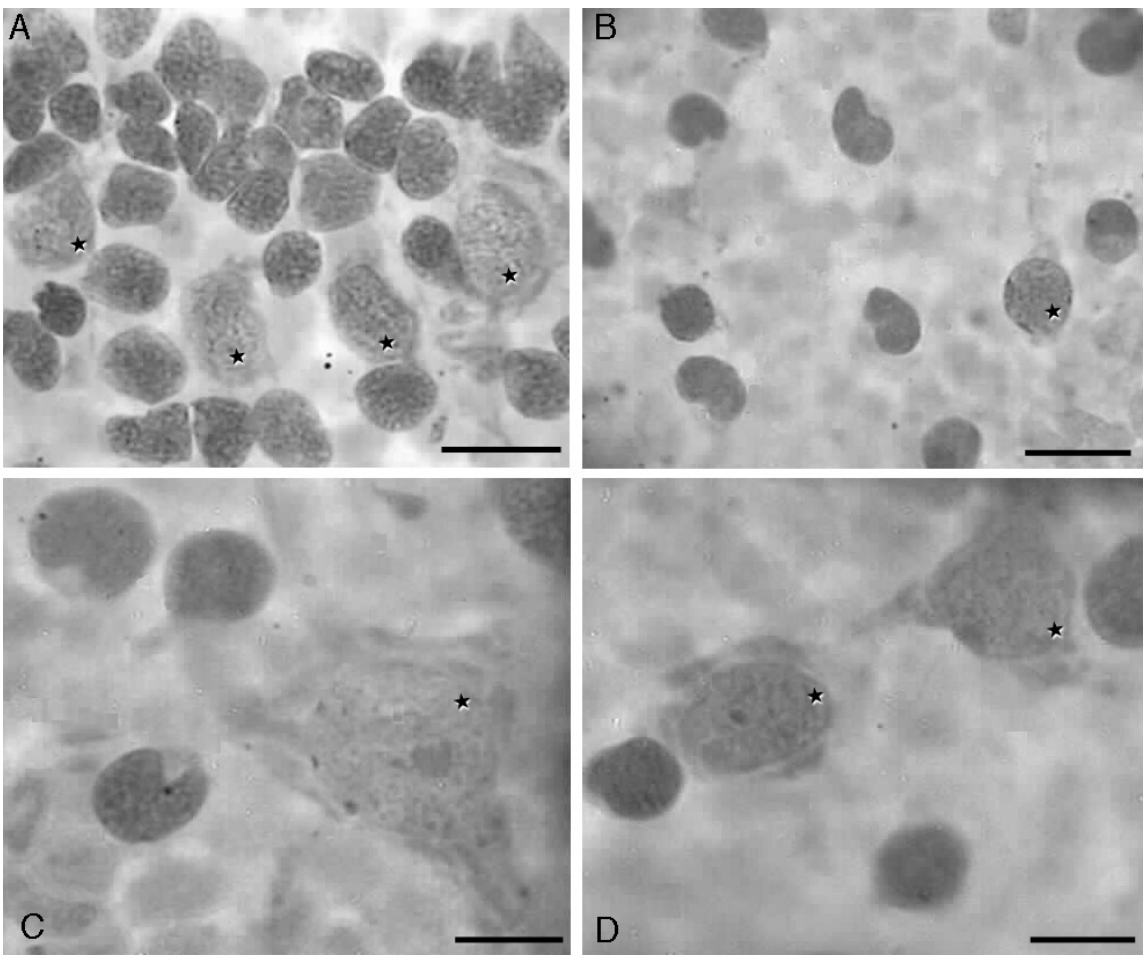

FIG. 4. – Light micrograph of cells in the ganglion cell layer in the retina of *C. coelolepis*. A, the close-fitting cells in the centro-caudal specialised area where large cells are indicated with an asterisk; B, low cell density in a non-specialised area outside the visual streak; C and D, large cells (asterisks) in the retinal periphery and in the specialised area at higher magnification. Scale bar = 10 μm.

cells constituted between 7 and 9% of the total cell population, although their dimension and density depended on their regional localisation in the retina. In the radial semithin sections of the retina, some of these cells were localised in the "scleral sublamina" of the IPL (Fig. 1), while other cells were found in the "vitreal sublamina". The iso-density distribution map was attempted for all these cells independently of their location. Interestingly, the distribution of these cells in the ganglion cell layer was very similar to that of the total cell population, forming elongated areas of maximum cell density in the same position as the horizontal meridian of the retina (Fig. 2 A' and B'). However, the peak density of the large ganglion cells did not match exactly the peak density of the total cell population. In fact, the specialisation of the centro-temporal retina showed a rather homogeneous population of small and medium-sized neurones (Fig. 3B and B').

The specialised area of higher visual acuity was located centro-temporally in both the studied individuals, but it was placed at 32º from the geometric centre of the retina and 5º above the horizontal meridian in the smaller fish and at 44º and 10º respectively in the larger one. This position indicated that the visual axis pointed in a slightly outward-forward direction with respect to the body axis of the fish (Fig. 5). In the two individuals, the peak density of large ganglion cells was located at around 2º and 4º respectively from the peak density of the total cell population.

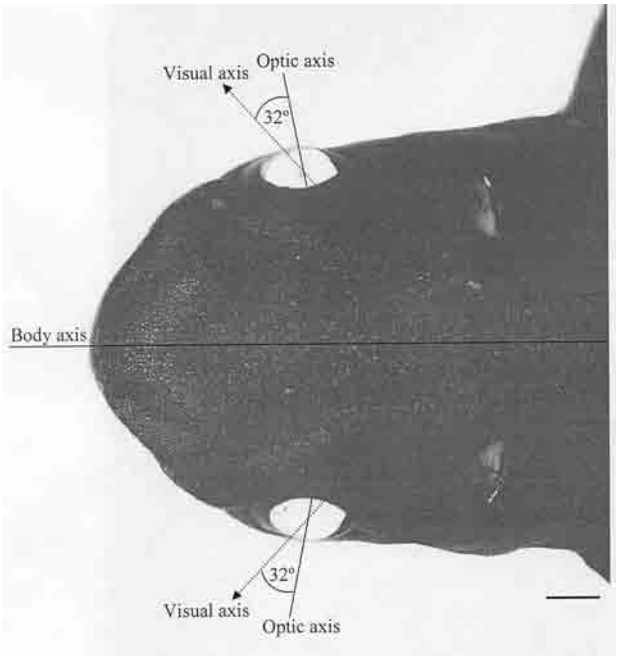

FIG. 5. – Dorsal view of the head of *Centroscymnus coelolepis* where the visual axis direction, positioned at 32º from the optic axis, has been shown. Scale bar = 1 cm.

DISCUSSION

The results of the present study reveal the visual adaptations of the Portuguese dogfish *Centroscymnus coelolepis* to a meso- and bathypelagic environment, corroborating the importance of vision for deep-sea sharks.

C. coelolepis exhibits a large eye, a large pupil and a spherical lens. A strong greenish-golden "eye shine" is indicative of the presence of a *tapetum lucidum*. The *tapetum*, common to several sharks from different environments (Denton and Nicol, 1964; Heath, 1991), is a specific device for increasing the amount of light absorbed by the photoreceptors that enhances visual sensitivity. In the tangential sections of the retina, the *tapetum* is visible in all areas, indicating the need for increased visual sensitivity in the deep-sea environment where light is uniformly scattered. The same *tapetum* characteristics have been observed in other deep-sea sharks such as *Etmopterus virens* (Gilbert, 1963) and *Galeus melastomus* (Bozzano *et al*. 2001). Recent analysis by Takei and Somiya (2002) of the *tapetum* and visual threshold in fish indicates that fish with *tapeta* could prey in a more extended space.

As many other deep-sea sharks, *C. coelolepis* has scotopic vision provided by a uniform population of rod-like photoreceptors, although the rods are not as long as expected for such a deep-sea species. In fact, the similar-sized *G. melastomus*, the second most abundant shark in the Mediterranean deep-sea, which is found down to a depth of 1700 m, shows rods almost twice as long as *C. coelolepis* (Bozzano *et al*., 2001). *G. melastomus* also has a 35% higher rod density than *C. coelolepis*. On the other hand, comparing the relationship between body length and eye and lens diameter in *G. melastomus* and *C. coelolepis* indicates that, for an equivalent fish length of 58 cm, *C. coelolepis* has the eye (15%) and lens diameter (19%) larger than *G. melastomus*. Large eyes and increased lens diameter magnify the image on the retina and, at the same time, improve visual acuity (Fernald, 1985). This would allow the studied species to widen the range of available prey and to be more selective. The theoretical visual acuity of *C. coelolepis* of 7.2-7.4 cycles per degree is higher than the acuity observed in *G. melastomus* (3.8) and in *E. spinax* (2.8), the third most abundant

dogfish in the deep Mediterranean, which is found down to 2000 m. Taking into account that the visual acuity in pelagic sharks (i.e. *Galeocerdo cuvieri* and *Negaprion brevirostris*) is 6.4 and 6.7 cycles per degree (Hueter, 1991; Bozzano and Collin, 2000), *C. coelolepis* therefore possesses a good theoretical visual acuity.

The ecological relationship between *C. coelolepis*, *G. melastomus* and *E. spinax* is quite interesting. The Mediterranean individuals of *C. coelolepis* dwell in deeper waters than the Atlantic and Pacific populations (Headrich and Merrett, 1988; Yano and Tanaka, 1984, 1988). Carrassón *et al*. (1992) postulated that this increase in depth distribution in the Mediterranean could be to avoid competing against other species with similar trophic habits, such as *Galeus melastomus* and *Etmopterus spinax*. Comparing the topography of retinal cells in the ganglion cell layer of *C. coelolepis* with the topography of *G. melastomus* and *E. spinax* described by Bozzano and Collin (2000), similar retinal distribution patterns can be observed, especially between *C. coelolepis* and *E. spinax*. Similarly to the latter species, the retinal isodensity contour map of *C. coelolepis* reveals a thin horizontal visual streak in the central plane of the retina that provides a panoramic field of vision that extends horizontally.

The specialised area located in the centro-temporal visual streak, and the visual axis pointing 32 and 44º from the body axis in the outward-forward direction, indicate a monocular visual field, but probably with a good perception of distant objects in the open marine environment. In a species like *C. coelolepis*, whose depth distribution range in the Mediterranean lies almost exclusively below the limit of daylight penetration where no visual horizon exists, it is surprising to find the need for visual horizon discrimination, which is characteristic of species that live in the epi- or mesopelagic environment (Collin and Pettigrew, 1988; Collin and Partridge, 1997; Bozzano and Collin, 2000). Thus, it is possible to hypothesise that the deeper distribution of this species in the Mediterranean is indeed a secondary adaptation in order to avoid competition with other species. In addition, this species might perform vertical migration. Although it has not been documented that any daily vertical migration of the studied species occurs, it was found that in *C. coelolepis* populations from both the north Atlantic (Clarke *et al*., 2002) and the Mediterranean (Crozier, 2001; Cló *et al*., 2002) gravid females tend to occur in shallower waters, while young individuals are reported at greater depths. Thus, the retinal specialisation for a better visual discrimination of the horizon might be useful when large females move to mesopelagic waters. As ontogenetic changes in the retinal ganglion cell topography have been documented in some teleosts (Shand *et al*., 2000; Bozzano and Catalán, 2002), determining possible changes in the cell distribution of the *C. coelolepis* retina during growth could help to increase the understanding of the migratory behaviour of this species.

The need for *C. coelolepis* to increase visual sensitivity in the bathypelagic environment is reflected in the high percentage of large ganglion cells (LGCs) that were found in the ganglion cell layer. Large ganglion cells, probably involved in motion perception (Collin *et al*., 1998), have been found in several teleosts from different habitats (Dunn-Meynell and Sharma, 1986; Cook and Becker, 1991; Cook *et al*., 1992; Bozzano, 2003), as well as in elasmobranchs (Gruber *et al*., 1963; Stell and Witkovsky, 1973; Anctil and Ali, 1974; Peterson and Rowe, 1980; Collin, 1988; Bozzano and Collin, 2000). These cells usually constitute a low percentage of the total cell population in the ganglion cell layer, but in deep-sea species their density can increase and/or form regional density variations. Specialised areas of large ganglion cells, defined as "areas *giganto cellularis*" were found in the tubular eyes of the deep-sea pearleye *Scopelarchus michaelsarsi* and *Scopelarchus analis* (Collin *et al*., 1998) and in the daggertooth deep-sea fish *Anotopterus pharao* (Uemura *et al*., 2000). Large ganglion cells were also found in *G. melastomus* and *E. spinax* (Bozzano and Collin, 2000). In *C. coelolepis*, LGCs were found throughout the retina. Although no "area *giganto cellularis*" was found, the large cells form a horizontal streak with elongated areas of increased density in the centre of the retina, which are very close to the peak density of the total cell population. This is the first time that a complete distribution map of large ganglion cells has been obtained in a shark, although a schematic representation of the concentration of giant ganglion cells was depicted by Collin (1988) in the shovel-nosed ray, *Rhinobatos batillum*. Though the mean LGCs soma size of *C. coelolepis* is larger than the giant ganglion cells of several sharks (except for *E. spinax*), their density in the specialised areas is much higher than the density found in the specialised areas of the retinae in *G. melastomus* and *E. spinax*. The morpholo-

gy of *C. coelolepis* LGCs was similar to that of the giant ganglion cells found in other elasmobranchs. As this morphology is analogous to the morphology of the α-cells reported in the cat retina (Wässle *et al.*, 1987), which were found to be related to increased sensitivity, this finding corroborates the necessity of this species to increase motion sensitivity especially in the horizontal field of view. The need to increase motion sensitivity can also be related to the thick inner plexiform layer. In fact, Buttery *et al.* (1991) observed better prey detection and improved movement processing in small mammalians that had a thick inner plexiform layer in the retina.

C. coelolepis is considered to be an active predator with a very specialised diet that feeds almost exclusively on cephalopods that live in the water column and, in general, on mobile organisms that have migratory habits (Roper, 1972). This indicates that vision has an important role in feeding behaviour and that prey detection and hunting may take place at different points in the water column. In addition, some deep-sea squid, on which the Portuguese dogfish prey, show strong bioluminescence. Bioluminescent prey found in the diet of *C. coelolepis* caught in the Mediterranean (*Heteroteuthis dispar* and *Histhioteuthis* sp.), as well as near South Africa (*Lycoteuthis lorigera*) and in the Atlantic (*Taningia danae*) (Clarke and Merrett, 1972; Carrassón *et al.*, 1992; Ebert *et al.*, 1992), confirm their preference for this type of prey. The spectral emissions of *H. dispar* photophores at 474 nm (Dilly and Herring, 1978) and of *T. danae* at 475-480 nm (Herring *et al.*, 1992) are well matched to the maximum absorption of the *C. coelolepis* visual pigment at 472 nm (Denton and Shaw, 1963). This overlap between the light emission of the prey and the maximum absorption of the visual pigment of the predator strongly suggests that bioluminescence is an important signal for *C. coelolepis* prey detection.

In conclusion, the Portuguese dogfish *C. coelolepis* has evolved ocular properties such as eye size, pupil area, lens size and tapetal reflection for increasing quantum capture in an environment where sunlight falls off completely and only bioluminescence is available. Furthermore, an increase of acuity along the horizontal visual field indicates an adaptation to an environment where a horizontal gradation of light within the water column exists. Finally, the high density and peculiar distribution of the large ganglion cells observed in the retina of this species seems to be related to its predatory behaviour since they allow this shark to perceive moving bioluminescent preys against a totally dark background.

Future research on the visual capabilities of *C. coelolepis* individuals from different oceans and depths could help to understand whether these visual adaptations are common to the species or, on the other hand, some of them could be specific to a segregated Mediterranean population, as suggested by Cló *et al.* (2002).

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