

Early development of eye and retina in lanternfish larvae

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(RECEIVED December 5, 2006; ACCEPTED April 30, 2007)

Abstract

The morphological characteristics of the eyes and the retinæ of lanternfish larvae of *Lampanyctus crocodilus*, *Benthoosema glaciale*, and *Myctophum punctatum* were analyzed in pre-flexion, flexion, and post-flexion stages. Pre-flexion larvae of *L. crocodilus*, the species with the shallowest depth distribution, had spherical eyes located antero-laterally on a strongly laterally-compressed head, suggesting a forward binocular visual field. *B. glaciale* and *M. punctatum* larvae live deeper in the water column and had eyes elongated in the dorsal-ventral plane. The eyes of *B. glaciale* were prominent, projecting slightly outward from a laterally-compressed head, suggesting a strongly laterally-directed visual field. *M. punctatum* had stalked elongated eyes projecting from a dorso-ventrally flattened head. The eyes can be freely rotated allowing lateral, anterior and dorsally-directed vision. A prominent choroidal gland was situated beneath the ventral portion of the eye in *M. punctatum* and *B. glaciale*, while a smaller gland was present in the dorsal and ventral portions of the eye of *L. crocodilus*. In pre-flexion stage larvae, the retina of all three species was differentiated with numerous rod photoreceptors in the peripheral retinal areas and fewer cone photoreceptors mainly distributed in the central retina. This distribution suggests concomitant enhancement of scotopic sensitivity in the vertical visual plane and improved photopic acuity in the lateral and forward visual directions. The concurrent development of cones and rods, as observed in the pre-flexion stage of myctophid larvae, is consistent with meeting the special demands of visual planktivory in sub-surface waters. During larval development a gradual increase of ROS length was also accompanied by a progressive loss of cones that were almost totally absent in post-flexion larvae. This can be interpreted as an adaptive response to an impending deep mesopelagic adult life.

Keywords: Larvae, Myctophidae, Eye shape, Retina, Development

Introduction

Most marine fish larvae hatched from small pelagic eggs are strongly visual predators at feeding onset (Blaxter, 1986; Pankhurst & Hilder, 1998; Rodríguez & Gisbert, 2002). Initially, the retinæ of these larvae are characterized by the exclusive presence of cone photoreceptors, limiting visual function to photopic conditions in near-surface waters, in which light intensities are relatively high (Blaxter, 1986; Powers & Raymond, 1990). It has been suggested that the small eye size of fish larvae constrains concurrent development of rod and cone photoreceptors, and that the ecological demands for visual planktivory results in optimization of acute visual resolution, a cone function, at the expense of sensitivity (Kotrschal et al., 1990). In these larvae, capacity for vision under scotopic conditions, or with increasing depth, develops with the subsequent development of rod photoreceptors (scotopic sensitivity) and with cone enlargement (photopic sensitivity). Increasing visual sensitivity during development of shallow water larvae frequently coincides with increasing depth distribution and change

in feeding ecology (Kawamura et al., 1984; Shand, 1997). In contrast to shallow water larvae, little is known of the visual ontogeny and ecology of deep-dwelling larvae.

In this respect, mesopelagic fish larvae belonging to the family Myctophidae (lanternfish) represent an extremely interesting example. Feeding ecology of most adult lanternfish involves daily vertical migrations, fish moving between 350 and 450 m in depth in order to feed in the sub surface-water layer (upper 100 m) at night (Sameoto, 1988; Hopkins & Gartner, 1992). In contrast, lanternfish larvae are presumed to be visual predators, feeding during the daytime (Sabatés et al., 2003), suggesting a marked ontogenetic shift in their feeding ecology and behavior.

Lanternfish larvae exhibit a high diversity of morphological traits (Moser, 1981), among which eye shape is one of the most important. Larvae fall into two groups on the basis of eye shape: those with narrow elliptical eyes (in some genera they are borne on stalks) and those with round or nearly round eyes (Moser & Ahlstrom, 1970, 1974). The species composition of these two groups of lanternfish agrees closely with that of the two subfamilies, Myctophinae and Lampanyctinae, established by Paxton (1972) on the basis of osteological and photophore characters of adults. It has been suggested that these differences in morphological (and likely physiological) characteristics of the eyes of

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lanternfish larvae might result in different feeding strategies (Sabatés & Saiz, 2000, Sassa & Kawaguchi, 2005).

In this study, larvae of two Myctophinae (*Myctophum punctatum* and *Benthosema glaciale*) and one Lampanyctinae (*Lampanyctus crocodilus*) species at the pre-flexion, flexion and post-flexion stages were investigated. The larvae of *L. crocodilus*, a species with round eyes, show a relatively shallow depth distribution, preferentially in the upper 30 m of the water column (Olivar & Sabatés, 1997). At these depths light intensity in the Mediterranean Sea at the end of summer can vary between 600 and 200 $\mu\text{mol s}^{-1}\text{m}^{-2}$ (Sabatés et al., 2003) at midday. The larvae of *B. glaciale* show an intermediate distribution between 20 and 100 m where maximum light intensity ranges between 200 and 1.3 $\mu\text{mol s}^{-1}\text{m}^{-2}$ while larvae of *M. punctatum* have a deep distribution in the water column between 50 and 150 m (Olivar et al., 1998; Sabatés, 2004), and a maximum available light between 22 and 0.1 $\mu\text{mol s}^{-1}\text{m}^{-2}$. The larvae of the two latter species have elliptical eyes, and in the case of *M. punctatum*, they are also borne on stalks. All three species are known to be visual predators as they feed during daylight hours and in addition to this, foraging strategies are reported to vary in relation to ambient light intensity (Sabatés et al., 2003).

Taking into account that the early ontogeny of fish larvae can be regarded as a trade-off between environmental abiotic factors (for example, light intensity and photoperiod), functional demands (feeding strategies and depth distribution) and developmental stages, in this study, it is hypothesized that such a relation between ecological needs and structural differentiations may exist in the eye of the lanternfish larvae.

Materials and methods

Field sampling and histological analysis

Lanternfish larvae were collected in the Western Alboran Sea (Western Mediterranean) during a research cruise conducted from May 2 to 16, 1998. The sampling was performed at three stations located in a transect perpendicular to the coast (36° 00' N - 36° 23' N, 4° 14' W - 4° 16' W). A different station was sampled each day, both during day and night. Fish larvae were collected with Bongo-type plankton net with a mouth opening 60 cm in diameter and a mesh size of 333 μm . The tows were carried out from a maximum depth of 200 m, at a ship speed of 3.7 km h^{-1} (2 knots).

Upon capture, a total of 54 individuals of lanternfish larvae of *L. crocodilus* ($n = 16$), *B. glaciale* ($n = 19$), and *M. punctatum* ($n = 17$) were separated on board and fixed overnight in a solution of 2.5% glutaraldehyde, 1% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 to which 3% sucrose was added in order to provide a fixative that was near-isotonic with seawater fish tissue and therefore reduced tissue shrinkage. In myctophid fish larvae shrinkage has been reported to be ca. 3–7% (Moku et al., 2001, 2004) with formalin seawater and ethyl alcohol freshwater fixatives. The use of sucrose in the fixative we employed, we assume to have reduced this value. Therefore, no correction was applied to larval fish measurements. After fixation, the samples were transferred to 0.1 M phosphate buffer and maintained at 4°C until reaching the laboratory. Larvae were classified into pre-flexion, flexion and post-flexion stages. Standard length (SL) was measured, using light microscopy (to the nearest 0.1 mm), from the tip of the snout to the end of the notochord in pre-flexion and flexion larvae, and from the tip of the snout to the posterior margin of the hypural plate in post-flexion larvae.

Dorso-ventral (DV) and rostro-temporal (RT) eye diameters were measured prior to embedding and the relative eye size (DV/SL and RT/SL) was calculated. In each developmental stage, the relationship DV/RT was used to calculate the elliptical index, (elliptical index of a spherical eye = 1).

Twelve individuals of each species were subsequently dehydrated in a graded ascending series of ethanol-water mixtures and infiltrated in plastic resin (Historesin-Leica) overnight. A total of four (five for pre-flexion stage) individuals from each development stage were oriented and embedded for transverse or horizontal sectioning. Serial sections (2–3 μm) were cut and stained with methylene blue and basic fuchsin (Lee's staining procedure, www.ebsciences.com). Cell morphometrics and measurements of the lens dimensions were taken in the central retina in sections with the largest lens diameter. Photoreceptor outer segment length and linear densities of photoreceptors and putative ganglion cell nuclei were measured along a 100 μm linear transect in the dorsal, central and ventral regions of the retina, away from the optic nerve and falciform process, using a light microscope and the Optimas 6.0 image analyzer. For the calculation of putative ganglion cell density, a prior cell somata analysis of the cells in the ganglion cell layer (GCL) was done. The cell soma areas were measured using the Optimas 6.0 image analyzer and the putative displaced amacrine cells (DACs), which do not contribute to visual acuity and that are known to be located within the GCL (Collin, 1988; Collin & Pettigrew, 1988; Wagner et al., 1998; Mack et al., 2004), were differentiated on morphological grounds according to criteria identified by Collin (1988). Small, circular, deep-stained somas were putatively associated with the DACs, while the large, granular, and irregularly shaped profiles were attributed to putative ganglion cells (GCs). Angular cell density was calculated for rods, cones and putative GCs, according to Poling and Fuiman (1998), including an adjustment for a potential error in linear ganglion cell densities associated with the angular curvature of small eyes. Visual acuity intended as minimum separable angle (MSA) was calculated using a modification of the equation given by Neave (1984) as proposed by Poling and Fuiman (1998). It is worth noting that there is a considerable morphological and functional diversity among GCs. Hirt and Wagner (2005) identified up to 10 different types of GCs in a deep sea fish and not all of them contributed to visual acuity. Thus, in the present study, ideally we should have at least distinguished between ON and OFF centre ganglion cells, but the ship-board sampling precluded this physiological approach. Therefore, the ganglion cell density we calculated contributing to the optic tectum input for acute visual resolution could be overestimated by 50%.

One individual of each development stage was used to examine retinal ultrastructure. After primary fixation onboard ship, the larvae were post-fixed in 1% osmium tetroxide and embedded in Spurr resin. Ultrathin sections (0.5–0.6 μm) in the transverse plane were stained with lead citrate and uranyl acetate and examined using a Hitachi 600AB transmission electron microscope TEM.

A Student's *t*-test was used to compare the effect of retinal region and larval growth on the rod-like photoreceptor length and density, as well as on the putative ganglion cells. The effect of retinal region on the cone-like photoreceptors was tested only in pre-flexion stage larvae, while no test was performed in flexion and post-flexion stages due to the non-uniform distribution of the cones. For each retinal region, interspecific changes in the rod-like photoreceptor length and density, as well as in putative ganglion cell density were also tested.

Results

Shape and position of the eye

At the pre-flexion stages, the eyecup of all three species appeared dark due to the retinal pigment epithelium (RPE) situated adjacent to the sclera. The lens and the cornea of all larvae bulged out of the eyecup. The position and orientation of the eyes in the head of each species are shown in Fig. 1.

In *L. crocodilus* larvae, the species with the shallowest depth distribution, the eyes were positioned on a strongly laterally compressed head and angled obliquely inward toward the snout (Fig. 1,

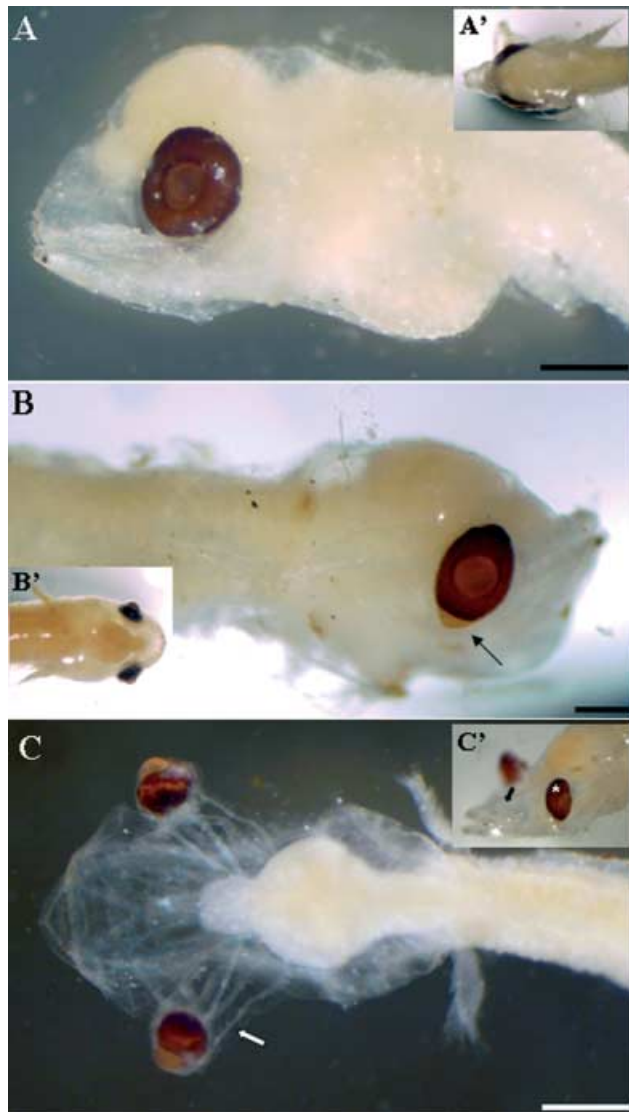


Fig. 1. Photographs of lanternfish larvae prior to flexion. (A) *Lampanyctus crocodilus* larva (5.04 mm SL) in lateral view showing a near-spherical eye. Inset: *L. crocodilus* (4.76 mm SL) viewed from above showing the lateral orientation of the eyes in the head. (B) *Benthosema glaciale* larva (5.2 mm SL) in lateral view showing an elliptical eye. The arrow indicates the choroidal gland. Inset: *B. glaciale* larva (7.04 mm SL) in dorsal view. (C) *Myctophum punctatum* larva (5.6 mm SL) in dorsal view showing the stalked (white arrow) eyes. Inset: *M. punctatum* larva (7.64 mm SL) in lateral view showing the elliptical eyes (asterisk), reduction of eyestalks and the dorso-ventrally flattened head (black arrow). Scale bar: 0.5 mm.

inset). They were proportionally larger than the eyes of the other two species and almost spherical (Fig. 1A, Table 1), with mean elliptical indices of 0.99 ± 0.1 and 0.87 ± 0.06 in pre-flexion and post-flexion larvae, respectively (Fig. 2). After the examination of all the serial sections of the entire eye, a small choroidal gland was observed in the dorsal and ventral portions of the eye (Fig. 3A). The eyes of *B. glaciale* larvae were more prominent than those of *L. crocodilus*, projecting further outward from the head and were less obliquely angled toward the snout (Fig. 1, inset). The eyes of this species were elongate in the dorso-ventral plane (Fig. 1B, Table 1) with a mean elliptical index of 0.69 ± 0.05 that remained fairly constant during larval development (Fig. 2). In addition, the eye had a pronounced ventral choroidal gland, especially noticeable in the pre-flexion stage (Figs. 1B, 3B). It appeared as a conical mass of highly vascularised conjunctive tissue. In *M. punctatum* larvae, the species with the deepest distribution, eyes were extended on stalks arising from the anterior region of the forebrain (Fig. 1C). They showed the smallest relative size especially in the rostro-temporal plane and were elongated in the dorso-ventral plane (Fig. 1, inset, Table 1) with a mean elliptical index of 0.53 ± 0.1 at the pre-flexion stage. Attenuation of the optic stalk occurred in the post-flexion stage coincident with a progressive rounding of the eye (elliptical index of 0.61 ± 0.06) (Fig. 2). A prominent choroidal gland located beneath the ventral portion of the eye (Fig. 3C) was also observed in this species.

Retinal morphometry

Pre-flexion larvae

At this stage, the retina was well developed in all three species of lanternfish and the eyes were presumed to be fully functional, as evident by differentiation of all retinal cell layers (Fig. 4), pigmentation of the RPE, crystallization of the lens (a result of water extrusion from cells and concentration of crystalline and protein fractions responsible for lens transparency and high refractive index; Nicol (1989) and the optic nerve contacting the optic tectum.

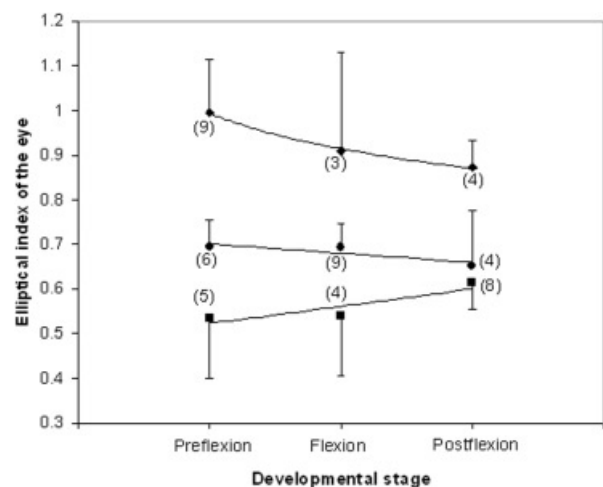


Fig. 2. Changes in the elliptical index of the eye during growth in *Lampanyctus crocodilus* (rhombus, $y = 0.99x^{-0.12}$, $r^2 = 0.99$), *Benthosema glaciale* (circles, $y = 0.72e^{-0.03x}$, $r^2 = 0.76$) and *Myctophum punctatum* (squares, $y = 0.49e^{0.07x}$, $r^2 = 0.80$). Values are means \pm SD; n values are provided in brackets beside mean values.

Table 1. Measurements of the eye and lens diameters in the three lanternfish larvae examined during each development stage. The relative eye size is expressed as the relation between the standard length (SL) and the DV (dorso-ventral) and RT (rostral-temporal) eye diameters. Lens diameter is expressed as mean \pm standard deviation. Depth range, standard length (SL) and number of examined individuals are also indicated

Species	Depth range* (m)	Developing stage	n	SL (mm)	DV/SL (%)	RT/SL (%)	Lens diameter (μ m)
<i>L. crocodilus</i>	<30 m	Pre-flexion	5	4.35–5.04	9.1	9.0	153.0 \pm 18.3
		Flexion	3	6.3–6.85	11.4	9.9	260.7 \pm 10.2
		Post-flexion	3	8.88–9.76	9.2	8.0	308.2 \pm 7.2
<i>B. glaciale</i>	20–100	Pre-flexion	5	4.2–5.48	8.8	6.7	169.4 \pm 22.6
		Flexion	2	7.2–7.6	7.7	6.2	229.1 \pm 17.3
		Post-flexion	2	8.44–8.88	7.9	5.7	242.6 \pm 5.0
<i>M. punctatum</i>	50–150	Preflexion	4	5.15–6.08	7.4	4.4	132.0 \pm 35.4
		Flexion	2	8–8.16	7.9	5.2	231.0 \pm 11.9
		Postflexion	3	9.7–13.7	6.8	4.8	277.4 \pm 30.6

*According to Olivar et al. (1998), Sabatés et al. (2003), and Sabatés (2004).

There were two types of photoreceptors evident in larvae of all species of lanternfish investigated: rod-like (presumptive rod) and cone-like (presumptive cone) photoreceptors (Fig. 5A). Presence of two morphologically distinct types of nuclei in a single-layered outer nuclear layer (ONL) supported this hypothesis. Identification of rod and cone photoreceptors was made in accordance with the morphological characteristics ascribed to each photoreceptor type (Ali & Klyne, 1985). The nuclei of presumptive cones were sparsely distributed, round, light-stained with spread chromatin,

whereas those of presumptive rods were more prevalent and dark-stained with concentrated chromatin (Figs. 5C–5E). A single pedicle-type synaptic process was observed in the outer plexiform layer (OPL) of the retina of all lanternfish larvae (Fig. 5B). Identification of a single synapse type may be a limitation associated with synapse differentiation at this developmental stage, and does not necessarily mean that only one type of synaptic process was present. In fact, it is possible that the classical morphological diagnostic characteristics of pedicles versus spherules are less

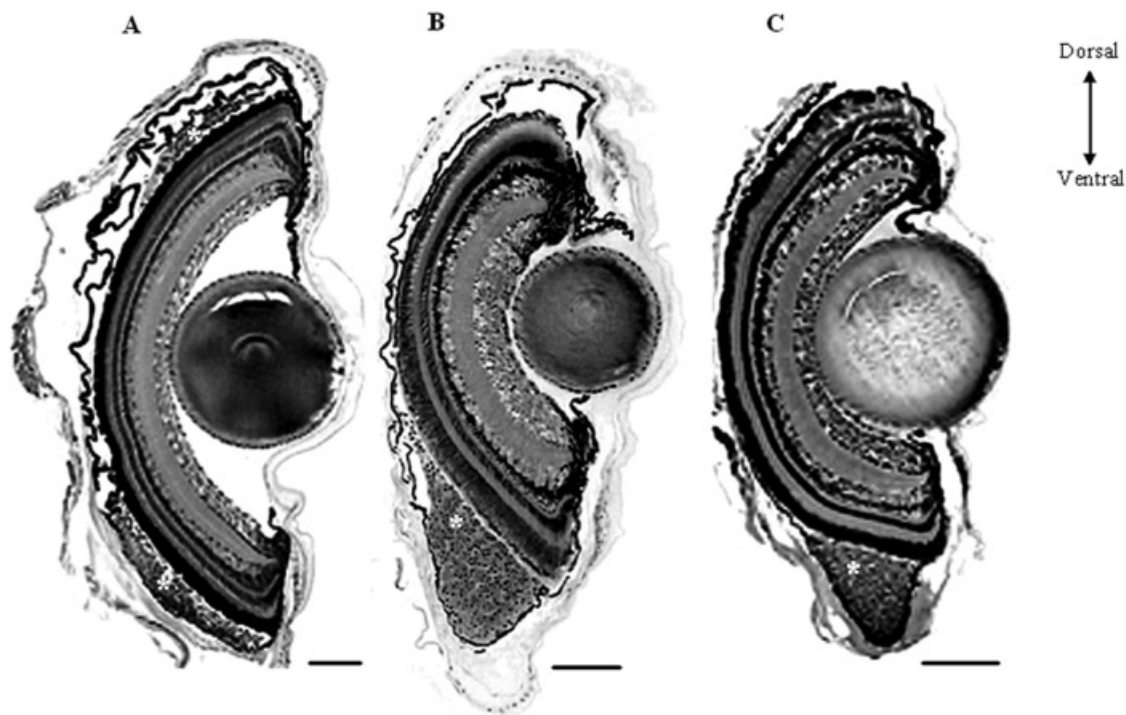


Fig. 3. Transverse sections of the eyes of lanternfish larvae in postflexion stages. (A) *Lampanyctus crocodilus* (9.5 mm SL), (B) *Benthosema glaciale* (7.6 mm SL), and (C) *Myctophum punctatum* (8 mm SL). Note the differences in the degree of development of the choroidal glands (asterisks). Scale bar: 100 μ m.

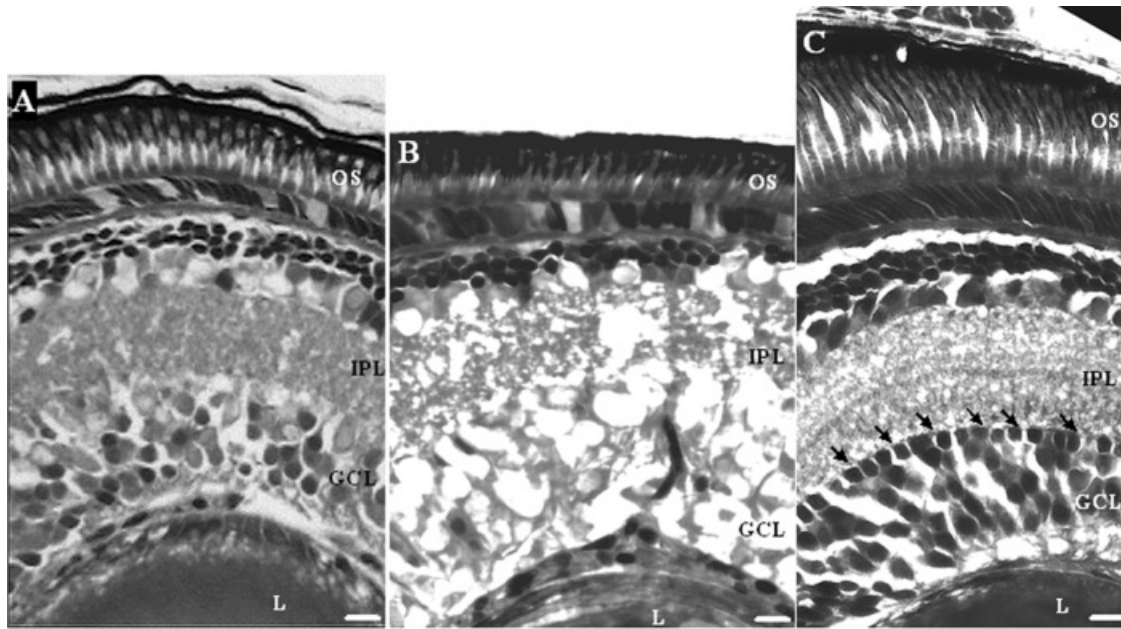


Fig. 4. Transverse sections of the retina of lanternfish larvae prior to flexion. (A) *Lampanyctus crocodilus* (4.4 mm SL), (B) *Benthosema glaciale* (4.3 mm SL), and (C) *Myctophum punctatum* (5.2 mm SL). L, lens; OS, photoreceptor outer segment; IPL, inner plexiform layer; GCL, ganglion cell layer. The black arrows indicate a row of putative displaced amacrine cells. Scale bar: 5 μm .

obvious at this stage. Rods had long slender outer segments (OSs) and TEM observations confirmed that the lamella discs within the OSs were discontinuous with the plasma membrane.

In the retina of *L. crocodilus* larvae, cones were observed mostly in the central retina (Table 2). The OSs were conical and the lamella discs appeared to be continuous with the plasma membrane. A cone accessory OS or “lateral sac,” was sometimes observed running parallel with the cone outer segment, and numerous calycal processes were visible in tangential sections around the cone OS circumference (Fig. 5A). The inner segment of the rods of all three species contained mitochondria of uniform dimensions. The lighter staining ellipsoid of the cone photoreceptors was filled with wider aggregations of mitochondria full of vacuoles (Fig. 6).

In pre-flexion larvae of the lanternfish species examined, there were significant differences in rod OS length ($p < 0.05$) in relation to retinal region, with rod OS length longer in the dorsal and ventral regions compared to the central retina (Table 2). There were no significant differences among species ($p > 0.05$). Angular densities of rods were uniformly higher than that of cones in all retinal regions examined (Table 2) and did not vary throughout the retinal areas in any species, with the exception of *M. punctatum*, in which it was significantly higher in the central retina (0.16 ± 0.01 cells 10 min arc^{-1}). Angular densities of cones were highest in the central retina in *L. crocodilus* (0.012 ± 0.01 cells 10 min arc^{-1}), more evenly distributed throughout dorsal, ventral, and central regions of the ONL in *B. glaciale* (0.02 ± 0.01 cells 10 min arc^{-1}), whereas they had a patchy distribution in the ONL of *M. punctatum*, with highest densities in the dorsal retina (0.013 ± 0.01 cells 10 min arc^{-1}) (Table 2). In all species, the putative displaced amacrine cells (DACs) constituted between 40 and 60% of the entire population of cells in the GCL and their soma was smaller than the putative ganglion cell soma (Figs. 4C, 7, Table 3). The summation ratio of rods: putative ganglion cells (GCs) was close to 2 in *L. crocodilus*, 2.5–4 in *B. glaciale* and ca. 1.5 in *M.*

punctatum. The summation ratio of cones: putative GC varied between 1.2–0.7 in *B. glaciale*, 0.1–0.03 in *M. punctatum* and 0.2–0.07 in *L. crocodilus* (Table 2).

Flexion larvae

The outer retina showed evidence of cellular reorganization, as evident by the highly irregular arrangement of photoreceptor nuclei in the ONL, that appeared to be related to the loss of cone photoreceptors, particularly in the dorsal and central areas of the retina in *L. crocodilus* (Fig. 8A) and in the ventral and dorsal regions in the retina of *M. punctatum* (Figs. 8B, 8E) and *B. glaciale* (Fig. 8C). At the ultrastructure level, while the plasma membrane of cones appeared to remain intact, cone OS lost their regular compact lamella structure, developing large inter-membrane spaces and separation of lamella discs (Figs. 8D, 8E). At the same time, mitochondria within the cone ellipsoids appeared to be degenerating, showing ultrastructural vacuolization (Figs. 8D, 8E).

Flexion larvae showed no increase in either outer segment length or angular density of rods; however, a decrease in the angular density of putative GCs was observed in *L. crocodilus* and *M. punctatum*. Displaced amacrine cells constituted ca. 40% of the cells population in the GCL in *B. glaciale* and *M. punctatum*, while they reached 68% in *L. crocodilus*.

Post-flexion larvae

Long rod photoreceptor (OSs) were still observed in the dorsal and ventral retina of *M. punctatum* and *B. glaciale*, while a more homogeneous OS length distribution was observed in the retina of *L. crocodilus* (Table 2). The areas of cellular reorganization attributed to cone loss were still present in the dorsal ONL of *L. crocodilus* and *M. punctatum* and in the central region of the ONL in *B. glaciale*; however these areas were much reduced compared to earlier developmental stages. In all the retinal regions, the ONL was characterized only by rods (Fig. 9). The angular density of the putative GCs decreased further in *L. crocodilus* and *M. punctatum*,

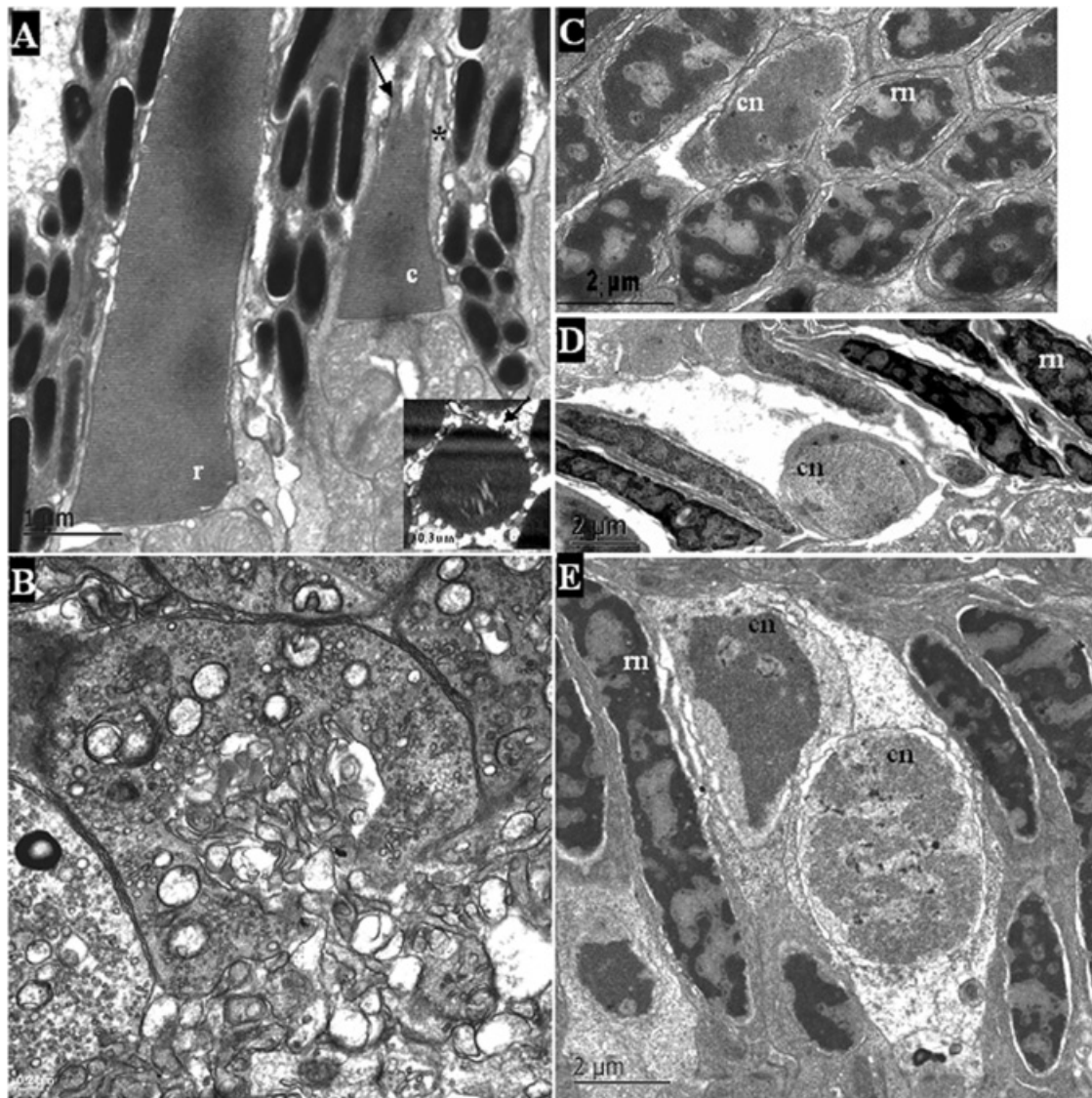


Fig. 5. Transmission electron micrographs of the retina of lanternfish larvae prior to flexion. (A) Outer segments of rod- (r) and single cone-like (c) photoreceptors in a *Lampanyctus crocodilus* larva (4.24 mm SL). Note the calycal processes (arrow) and the lateral sac (asterisk) of the presumptive cone outer segment. Inset: Tangential section of a putative cone showing numerous calycal processes (arrow). (B) Presumptive cone synaptic terminal in a *Myctophum punctatum* larva (6.12 mm SL). (C, D, and E) presumptive rod (m) and cone (cn) nuclei in retinas of *L. crocodilus*, *B. glaciale* and *M. punctatum* larvae, respectively.

while it maintained fairly constant in *B. glaciale*. Analysis of the summation ratio of rods: putative ganglion cells revealed that in *B. glaciale* and *M. punctatum* this relationship remained fairly constant, while in *L. crocodilus* it increased in all the retinal regions. The number of DACs increased only in *L. crocodilus* reaching 78% of the total cell population in the GCL, while in the other two species at this stage this value maintained practically constant (Table 3).

Discussion

Preflexion stage

Ontogeny of the larval eye for all three lanternfish species investigated involved changes in morphology of the retina and eye

shape. Pre-flexion larvae had duplex retinæ dominated by rod-like photoreceptors. The rod-like and cone-like photoreceptors found in the retinæ of these species showed a number of ultrastructural features which they share with photoreceptors of other teleost larvae (Ali & Klyne, 1985) and therefore they will be referred to as rods and cones. The presence of rods at this early stage of larval development is contrary to that reported for larvae of most shallow water marine fish, in which the retina is initially characterized by the exclusive presence of cones, rods only appearing later, close to, or at metamorphosis (Sandy & Blaxter, 1980; Kawamura et al., 1984; Powers & Raymond, 1990; Pankhurst & Eagar, 1996; Shand 1997; Matsuoka, 1999; Shand et al., 1999a; Lara, 2001). In contrast, larvae of the Japanese eel, *Anguilla japonica* (7 days old reared larvae and 11.0 mm wild caught leptocephalus, ca. 2 weeks post-hatching) and of the deep sea sawtail fish *Idiacanthus fasci-*

Table 2. Summary of the measurements of rod outer segment (ROS) length, and rod, cone and putative ganglion cells (GC) angular densities in different retinal regions during the lanternfish larvae development. Visual acuity (MSA) and summation ratios are also shown

Species		Photoreceptors outer segment length (μm)			Rod angular density (cells 10 min arc^{-1})			Cone angular density (cells 10 min arc^{-1})			Putative GC angular density (cells 10 min arc^{-1})		
		Dorsal	Central	Ventral	Dorsal	Central	Ventral	Dorsal	Central	Ventral	Dorsal	Central	Ventral
<i>L. crocodilus</i>	Pre-flexion	24.2 \pm 4.5	12.8 \pm 2.1	21.9 \pm 1.1	0.118 \pm 0.01	0.130 \pm 0.02	0.131 \pm 0.02	0.004 \pm 0.01	0.012 \pm 0.01	0.001 \pm 0.01	0.074 \pm 0.03	0.087 \pm 0.05	0.077 \pm 0.02
	Flexion	26.0 \pm 5.5	15.9 \pm 5.1	21.1 \pm 1.5	0.107 \pm 0.01	0.096 \pm 0.01	0.096 \pm 0.01	0.002 \pm 0.01	0.022 \pm 0.01*	—	0.028 \pm 0.02	0.023 \pm 0.01	0.035 \pm 0.01
	Post-flexion	33.0 \pm 3.7	22.0 \pm 1.9	26.1 \pm 2.3	0.085 \pm 0.01	0.104 \pm 0.01	0.090 \pm 0.01	0.001 \pm 0.01	—	—	0.025 \pm 0.01	0.016 \pm 0.01	0.018 \pm 0.01
<i>B. glaciale</i>	Pre-flexion	29.4 \pm 4.7	10.6 \pm 3.4	23.0 \pm 6.6	0.100 \pm 0.02	0.110 \pm 0.05	0.098 \pm 0.02	0.023 \pm 0.01	0.022 \pm 0.02	0.016 \pm 0.01	0.042 \pm 0.02	0.067 \pm 0.06	0.064 \pm 0.04
	Flexion	28.7 \pm 3.7	20.5 \pm 2.3	30.2 \pm 2.2	0.108 \pm 0.02	0.134 \pm 0.01	0.096 \pm 0.02	—	—	0.015 \pm 0.02	0.060 \pm 0.01	0.087 \pm 0.02	0.057 \pm 0.01
	Post-flexion	27.9 \pm 2.0	19.4 \pm 1.0	25.2 \pm 0.2	0.112 \pm 0.01	0.115 \pm 0.01	0.110 \pm 0.01	—	0.011 \pm 0.01	—	0.066 \pm 0.01	0.077 \pm 0.01	0.061 \pm 0.04
<i>M. punctatum</i>	Pre-flexion	29.7 \pm 2.0	18.4 \pm 3.6	24.1 \pm 9.1	0.126 \pm 0.03	0.158 \pm 0.01	0.142 \pm 0.01	0.013 \pm 0.01	0.002 \pm 0.01	0.003 \pm 0.01	0.113 \pm 0.02	0.107 \pm 0.01	0.096 \pm 0.02
	Flexion	30.5 \pm 2.9	18.9 \pm 0.7	24.0 \pm 1.5	0.106 \pm 0.01	0.139 \pm 0.03	0.101 \pm 0.02	—	0.003 \pm 0.01	0.004 \pm 0.01	0.078 \pm 0.01	0.080 \pm 0.01	0.081 \pm 0.01
	Post-flexion	32.5 \pm 4.7	23.6 \pm 2.9	29.0 \pm 1.1	0.097 \pm 0.10	0.138 \pm 0.01	0.123 \pm 0.01	0.005 \pm 0.01	—	—	0.070 \pm 0.02	0.054 \pm 0.02	0.042 \pm 0.01

Species		MSA (degrees)			Summation ratio Rods: putative GC			Summation ratio Cone: putative GC		
		Dorsal	Central	Ventral	Dorsal	Central	Ventral	Dorsal	Central	Ventral
<i>L. crocodilus</i>	Pre-flexion	30.7 \pm 4.9	6.0 \pm 1.0	16.7 \pm 0.0	1.7	2.0	1.7	0.1	0.2	0.07
	Flexion	22.2 \pm 8.8	0.8 \pm 0.8	—	7.2	4.9	2.9	0.09	1.4*	—
	Post-flexion	31.1 \pm 0.0	—	—	3.8	5.7	8.0	0.06	—	—
<i>B. glaciale</i>	Pre-flexion	1.4 \pm 1.2	1.8 \pm 2.4	2.6 \pm 1.9	4.0	2.5	3.5	1.13	1.19	0.7
	Flexion	—	—	0.3 \pm 0.0	1.8	1.5	1.7	—	—	0.6
	Post-flexion	—	0.6 \pm 0.0	—	1.7	1.5	2.5	—	0.3	—
<i>M. punctatum</i>	Pre-flexion	24.8 \pm 4.8	42.6 \pm 4.4	7.5 \pm 0.0	1.2	1.5	1.5	0.1	0.03	0.08
	Flexion	—	4.7 \pm 0.0	2.6 \pm 0.0	1.4	1.8	1.2	—	0.07	0.1
	Post-flexion	0.8 \pm 0.0	—	—	1.5	2.8	3.0	0.06	—	—

*These values are due to a large confused area found in one individual where cone nuclei were still present but the OS were degenerating.

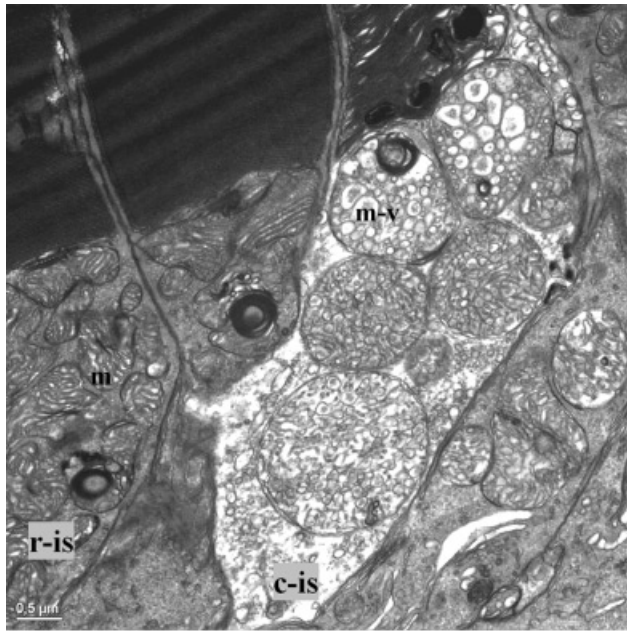


Fig. 6. Transmission electron micrographs of the rod-like (r-is) and cone-like (c-is) photoreceptors inner segments of *Myctophum punctatum*. Rod ellipsoids are filled with mitochondria while cone ellipsoid is characterized by the presence of both mitochondria full of vacuoles (m-v).

olatus were reported to have short cone-like photoreceptors in the centre of an otherwise rod-dominant retina (Omura et al., 1997; Wagner et al., 1998). In the present study, similar retinal morphology was observed in the pre-flexion lanternfish larvae investigated. On the other hand, the present study appears to be at odds with an early study by Pankhurst (1987) in which a pure rod retina was reported in a single larval specimen (5 mm TL) of the myctophid *Lampanyctodes hectoris*, which mainly dwell in the upper levels of the water column (0–20 m depth) (Olivar et al., 1992). However, the author acknowledged that in the absence of ultrastructural confirmation of photoreceptor type, identification of the photoreceptors as rods was open to question.

Early development of a rod-dominated retina is consistent with visual function under scotopic (dim light) conditions. While depth distribution of larvae of the three species of myctophids examined ranges from near-surface waters in *L. crocodilus* (<30 m), to 150 m in *M. punctatum*, scotopic sensitivity of pre-flexion larvae, as evident by the retinal morphological measurements of light path-length for photochemical stimulation (ROS lengths), angular densities of rods and summation ratios (rods: putative GCs), did not differ much between species, with the exceptions of *M. punctatum* with significantly higher angular density of rods in the central retina and *B. glaciale* with higher summation ratios in the dorsal and ventral retina. This certain overlap in their visual ecology is further supported by the consistent pattern in pre-flexion larvae of all three myctophid species of significant differ-

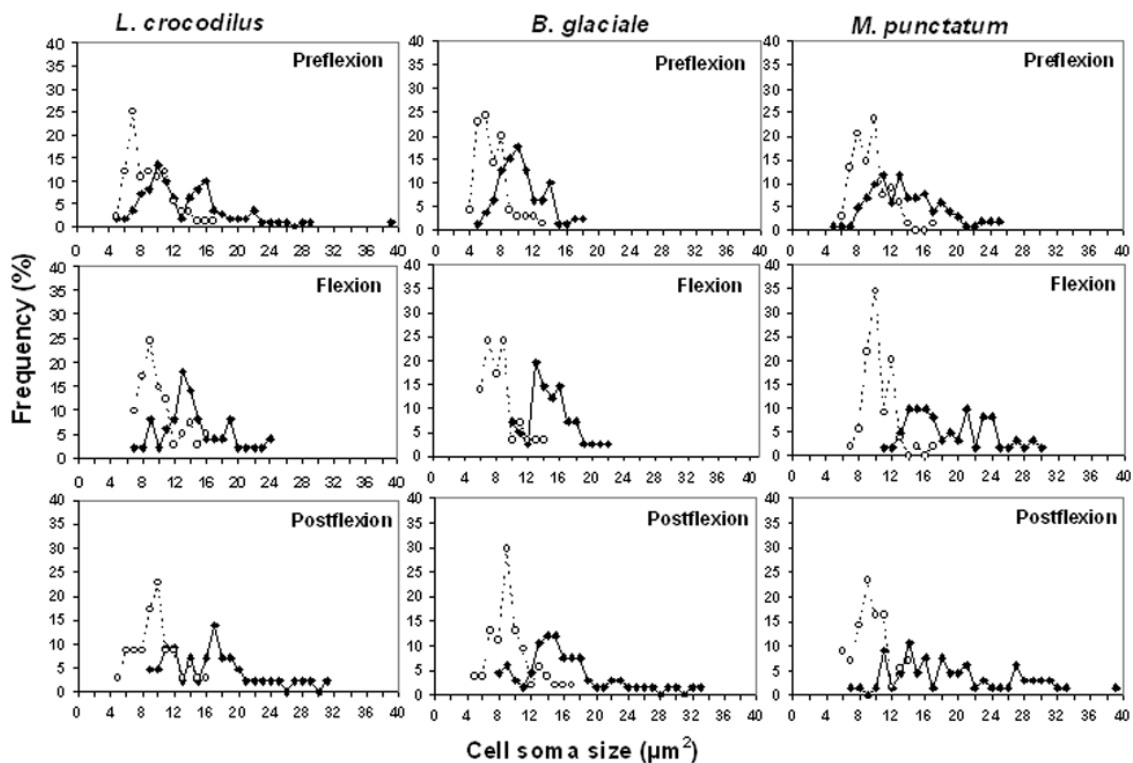


Fig. 7. Soma area versus frequency histograms of cell somata located within the GCL. The number of soma measures and the mean and standard deviation are listed in Table 3. Due to the relative low number of cells in the GCL, no matter was attended to discriminate between retinal areas.

Table 3. Summary of the mean soma size (μm^2) and standard deviation (SD) of the cell population lying within the ganglion cell layer (GCL), of the putative ganglion cells (GC) and displaced amacrine cells (DAC). In brackets is indicated the DAC percentage relative to the total cells in the GCL

	<i>L. crocodilus</i>			<i>B. glaciale</i>			<i>M. punctatum</i>		
	Cells in the GCL	Putative GC	Putative DAC	Cells in the GCL	Putative GC	Putative DAC	Cells in the GCL	Putative GC	Putative DAC
Pre-flexion									
<i>n</i>	189	101	84 (44%)	199	108	124 (62%)	149	79	60 (41%)
Size (μm^2)	12.1	14.4	9.5	11.7	13.7	9.3	9.3	11.0	7.4
SD	4.3	4.4	2.1	4.9	5.5	2.5	3.1	2.8	2.0
Flexion									
<i>n</i>	114	60	78 (68%)	88	48	38 (43%)	69	40	28 (42%)
Size (μm^2)	15.5	19.8	10.8	12.8	14.7	10.5	12.5	15.1	8.9
SD	5.8	4.9	1.7	3.9	3.8	2.4	3.9	2.7	2.0
Post-flexion									
<i>n</i>	118	62	92 (78%)	74	40	30 (41%)	115	63	48 (42%)
Size (μm^2)	15.3	20.1	10.0	13.7	16.7	10.1	13.3	16.3	9.7
SD	7.8	7.9	2.1	5.2	4.9	2.4	5.4	5.4	2.3

ences in rod OS lengths with retinal region, suggesting enhanced scotopic sensitivity in the dorsal and ventral planes compared to the central retinal regions.

Considering that the two species show an overlap in depth distribution, a difference of 50 m in the lower depth range means a difference of one order of magnitude in light intensity at midday ($1.3 \mu\text{E s}^{-1} \text{m}^{-2}$ at 100 m depth vs. $0.10 \mu\text{E s}^{-1} \text{m}^{-2}$ at 150 m) (Sabatés et al., 2003). In addition, the latter authors reported that larvae of both species showed distinct diel foraging patterns in relation to light intensity. In the case of *M. punctatum* larvae, feeding activity occurs mainly at dawn and dusk when light intensity can reach the threshold sensitivity ($1.85 \cdot 10^{-3} \mu\text{E s}^{-1} \text{m}^{-2}$) for larval fish feeding proposed by Blaxter (1986).

Given the early development of rods, it is not surprising then that rod densities found in the pre-flexion stage of myctophid larvae were similar to those observed in larvae of other pelagic fish close to metamorphosis. For example, linear rod densities between 35 and 45 cells $100 \mu\text{m}^{-1}$, corresponding to an angular density of 0.13–0.16 cells per 10 min of arc, were consistent with those calculated in postflexion or premetamorphic larvae of *Solea solea* (Sandy & Blaxter, 1980), *Perca fluviatilis* (Guma'a, 1982), *Forsterygion varium* (Pankhurst et al., 1993), *Pagrus auratus* (Pankhurst & Eagar, 1996) and of three species of sciaenids (Poling & Fuiman, 1998). Since rod photoreceptors are mainly associated with vision in low light intensities, the results may explain that light intensity thresholds for rod stimulation could be at least as important as other physical and biological variables in determining the taxonomic differences in depth distribution of these study species. In fact, the highest rod density calculated in the present study was in the larvae of *M. punctatum*, the species with the deepest distribution. Vertical zonation of fish larvae in relation to light intensity was also explained by Blaxter (1988), Leis (1991), and Job and Bellwood (2000).

At the pre-flexion stage, photopic visual acuity of lanternfish larvae was poor and varied greatly among species. For example, the best visual acuity calculated in the dorsal area of the retina of *B. glaciale* larvae, the species with the highest cone density among the studied species, was 1.4° MSA while visual acuity in *L. crocodilus* and *M. punctatum* were 6° and 7.5° MSA, respectively. Notwithstanding the limitations already stated in relation to esti-

mates of visual acuity in the present study, these values were fairly consistent with visual prey detection by other planktivorous larvae. In fact, anatomical values of $4\text{--}6^\circ$ MSA were calculated in the larvae of *Alosa pseudoharengus*, *Perca fluviatilis*, and *Clupea harengus* (Blaxter & Jones, 1967; Guma'a, 1982; Miller et al., 1993), while values of ca. $1\text{--}2^\circ$ MSA were instead obtained in larvae of *Premnas biaculeatus*, *Stethojulis strigiventer*, *Pleuronectes platessa*, *Scophthalmus maximus*, *Micropogonias undulatus*, *Sciaenops ocellatus*, *Cynoscion nebulosus* (Job & Bellwood, 1996; Margulies, 1997; Neave, 1984; Poling & Fuiman, 1998; Shand, 1997). Acuity of less than 1° MSA ($52'$) was calculated for larvae of *Pagrus auratus* (Pankhurst, 1994). Thus, theoretical acuities determined for first feeding fish larvae can range from 50 min of arc to $6\text{--}7^\circ$ MSA.

However, visual acuity reported for *M. punctatum* in the other retinal areas was inconsistent compared with the values indicated above for shallow water larvae.

In larvae of the three lanternfish species studied eye shape and position, along with body morphology, may be related with feeding strategies. Lanternfish larvae at these developmental stages are planktivorous and are assumed to be visual feeders, as their feeding activity is restricted to daylight hours (Sabatés et al., 2003). However, a contribution by non-visual senses to prey detection, especially in *M. punctatum* that show a quite low visual acuity, cannot be discounted as fish larvae frequently employ more than one sensory modality in prey detection (Pankhurst, 2007).

It has been reported that myctophid fish larvae exhibit a variety of feeding habits related to their morphological diversity (Sabatés & Saiz, 2000; Sassa & Kawaguchi, 2005). Body morphology of *L. crocodilus* larvae, with a deep body, laterally compressed head and large-toothed jaws (Moser et al., 1984) supports active pursuit of prey. In addition, the present study determined that the eyes of *L. crocodilus* were near spherical, located in an antero-lateral position and strongly angled inward toward the snout. The relatively large eye size that confers better acuity (because a given image is projected onto more photoreceptors) and presence of cone photoreceptors, mainly distributed in the centro-dorsal region of the retina, are consistent with a forward attacking visually mediated pursuit predator with binocular vision (Blaxter, 1965; Hobson & Chess, 1986; Cobcroft & Pankhurst, 2003, 2006). These characteristics are also

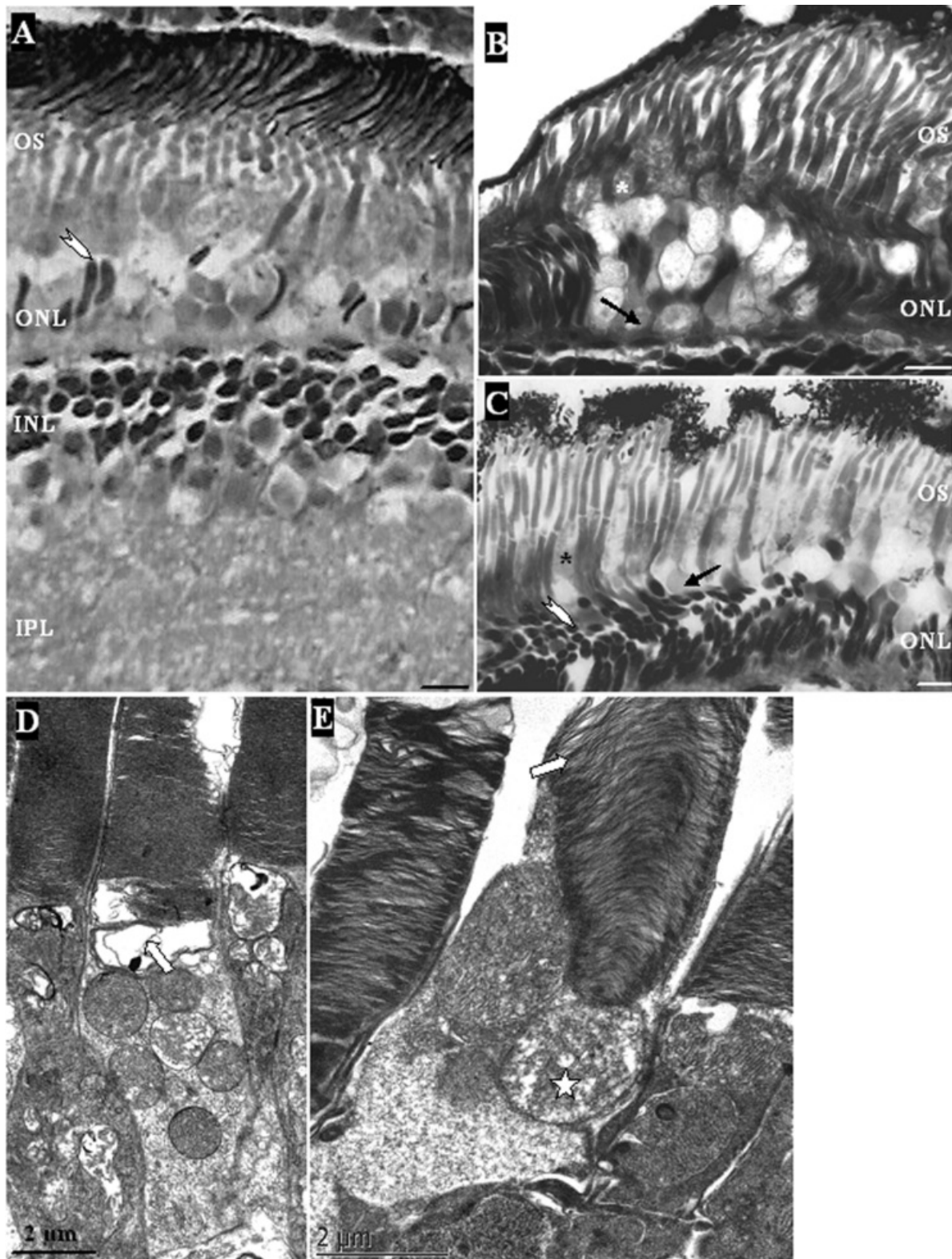


Fig. 8. (A–C) Light micrographs of the retinal outer nuclear layer in lanternfish larvae. The layer appear to be undergoing loss of presumptive cone nuclei (arrows) and inner segments (asterisk). In A most of the outer nuclear layer is constituted by degenerating nuclei and only few normal nuclei (white arrow) can be observed. In B a patch distribution of degenerating nuclei is observed (black arrow), while in C the degenerating cells are more regularly intercalated with the normal cells. (D–E) Transmission electron micrographs of the inner segments of the photoreceptors where cone OS lost their regular compact lamella structure, developing large inter-membrane spaces and separation of lamella discs (white arrows). Mitochondria also suffer degenerating process (white star). A and D, *Lampanyctus crocodilus* (6.75 mm SL); B and E, *Myxophum punctatum* (8 mm SL); C, *Benthosema glaciale* (7.6 mm SL). OS, outer segment; ONL, outer nuclear layer; INL, inner nuclear layer; IPL, inner plexiform layer. Scale bar (A–C): 6 μm.

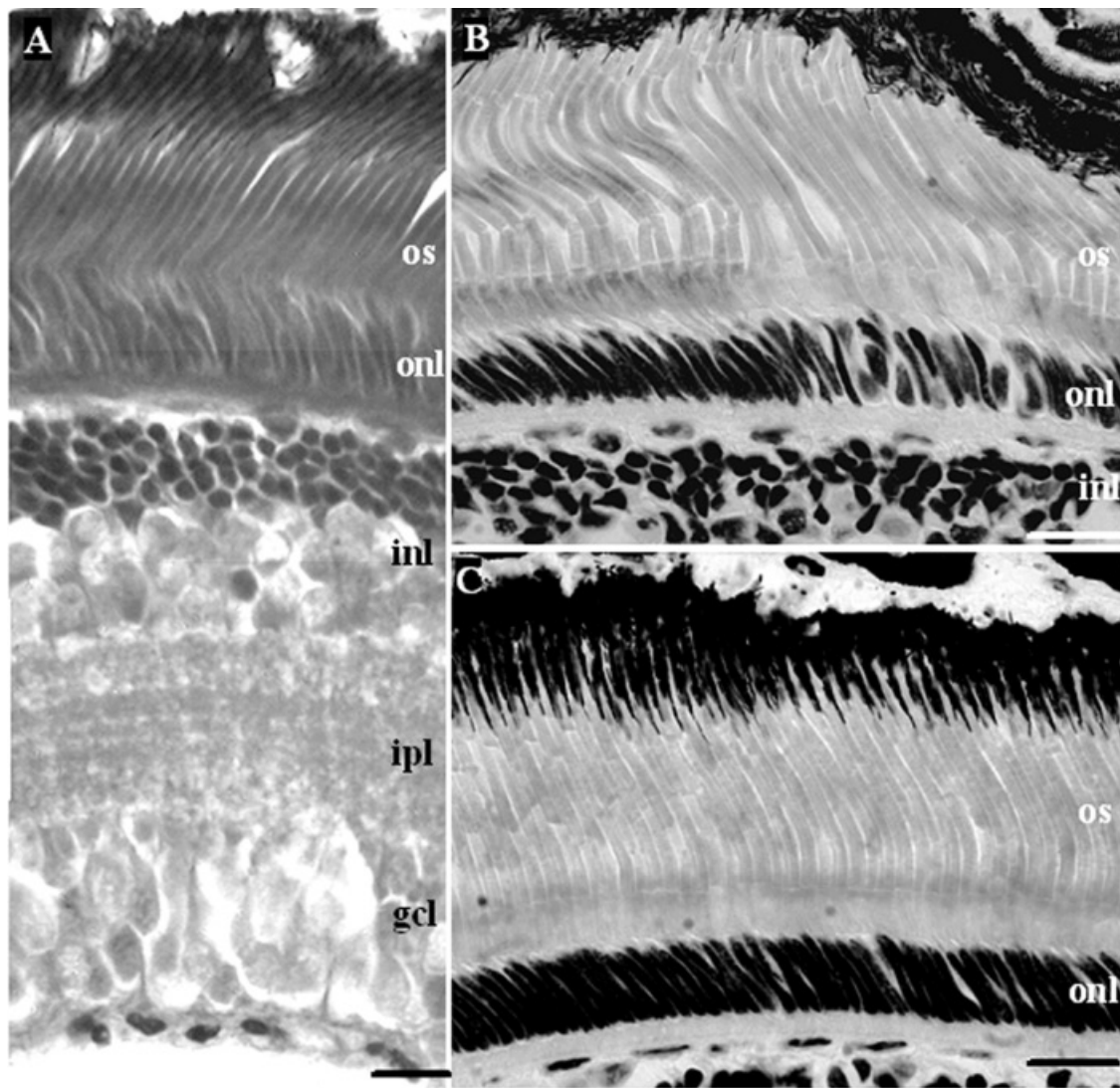


Fig. 9. (A) Light micrographs of the monolayered rod retinas of *L. crocodilus* (A), *B. glaciale* (B) and *M. punctatum* (C) in post-flexion stage. Os, outer segment; onl, outer nuclear layer; inl, inner nuclear layer; ipl, inner plexiform layer; gcl, ganglion cell layer. Scale bar: 10 μ m.

consistent with reports that larvae of this species feed during daytime in near-surface water (Sabatés & Saiz, 2000).

The larvae of *B. glaciale* occupy a relatively wide range of depths (between 20 and 100 m) compared to *L. crocodilus* larvae. Their body is fusiform, the mouth upturned and wide-spaced laterally-located elliptical eyes, suggest a lateral visual field extending in both dorsal and ventral planes. The combination of a dispersed array of cones, mainly in the centre of the retina, and the predominance of long rods in the periphery of the retina may confer on this species the ability to feed by visual means across its depth distribution throughout the daytime, as has been reported (Sabatés et al., 2003). The presence of cones suggests that acute visual perception is still important in preflexion larvae of this species when photopic conditions prevail, whereas rods may subserve prey detection, particularly in the dorsal and ventral visual fields, under scotopic conditions.

Pre-flexion larvae of *M. punctatum*, the species with the deepest distribution (50–150 m), have elongated bodies, wide mouths and broad dorso-ventrally flattened heads supporting small and

strongly elliptical eyes borne at the end of stalks. These characteristics are shared with larvae of other species inhabiting relatively deep water including Bathylagidae and Idiacanthidae families (Weihs & Moser, 1981; Moser et al., 1984). Lack of skeletal reinforcement in the eye stalks of *M. punctatum* larvae support our contention that these eyes can be freely rotated, potentially providing unimpeded vision in the lateral anterior and even dorsal planes and supports a conclusion by Weihs and Moser (1981) that stalked eyes of fish larvae allow a greater volume of water to be searched. An alternative and possibly not exclusive hypothesis, proposes that widely spaced eyes in larvae such as these reduce visibility to predators, the eyes being the most visible body feature (Johnsen, 2000). Similarly to *B. glaciale*, the elliptical nature of the eyes of *M. punctatum* would extend its visual field in the elongate axis. This body form, particularly the dorso-ventrally flattened head and mobile stalked eyes, seems to be consistent with an ambush predation strategy. If larvae hold the body horizontal with eyes projected upwards, this would presumably provide the possibility for larvae to detect prey passing overhead, silhouetted

against the predominantly down-welling space light, as has been reported for other mesopelagic ambush predators (Locket, 1977). In comparison to the other myctophid species examined, cone density and acuity were lowest in preflexion larvae of *M. punctatum*. This and the associated domination of rods in the retina support visual function predominantly under scotopic conditions which is consistent with their depth profile and field studies that report their maximum feeding activity periods to be restricted to twilight (dawn and dusk) (Sabatés et al., 2003). Presumably if non-visual senses predominated in prey detection by this species then feeding periodicity would be expected to be less truncated.

Flexion and Post-flexion stages

In all three species of lanternfish examined, notochord flexion was accompanied by rapid development of the fin rays, changes in larval body shape and locomotive ability, like other fish larvae (Kendall et al., 1984). Unlike larvae of shallow water species that at this stage incorporate rod photoreceptors into the retina, the retinæ of the lanternfish larvae were characterized by a dramatic decrease of cone density that almost disappeared in the late larval stage. Cones were also reported to disappear in the retina of leptocephalus stage *Anguilla japonica* (≥ 21.3 mm) (Omura et al., 1997), while pure rod retinæ were reported in early life history stages of some mesopelagic fish, including the leptocephalus stage of *Anguilla anguilla* (≥ 50 mm) (Pankhurst, 1984), late stage larvae of a macrurid species (≥ 17 mm SL) (Blaxter & Staines, 1970) and early juvenile viperfish *Chauliodus sloani* (≥ 29 mm SL; Locket, 1980). Probably decrease and disappearance of cones observed in lanternfish larvae in advanced developmental stages occurs along with a general preparation for a change to a deep mesopelagic habitat. In fact, as the cones density declines, a progressive increase in the rod OS length was observed, especially in the dorsal retina, presumably with concomitant increase in rod photosensitivity. A positive correlation between the depth of occurrence and the length of the photoreceptor outer segments was also found in several species of adult mesopelagic fish (e.g., Locket, 1980; Pankhurst, 1987; Wagner et al., 1998). The increased summation ratio of rods: putative GCs observed in the retina of *L. crocodilus*, as well as the almost doubling of the DACs with respect to previous developmental stages also suggest a further adaptation process of the retina to the impending mesopelagic adult life. While the DACs role in the GCL is still unclear, it has been observed that the 80% of the total neural population in the GCL in the retina of adult lanternfish *Lampanyctus macdonaldi* was constituted by DACs (Wagner et al., 1998). It has been suggested by Mack et al. (2004) that, because of the proximity of DACs to GCs, the DACs can exert strong influence on the output activity of the GCs, on one side inhibiting the response from GCs (Nirenberg & Meister, 1997), and on the other improving the detection of objects moving into the visual field of the eye. As the adults of *L. crocodilus* as well as *L. macdonaldi* can dwell in waters as deep as 1000–1200 m, an increase in movement detection can be useful in the predator-prey relationship.

During larval development, eye shape and position continue changing. The eyes of *B. glaciale* and *M. punctatum* became progressively rounded and the choroid gland reduced. In *M. punctatum*, both processes were also associated with an attenuation of the eye stalks and the final location of the eyes in the antero lateral orbits situated within the neurocranium. Rounding of the eyes and eyestalk absorption are processes likely to be associated with a significant metabolic oxygen demand that may indicate a role of

the choroid glands. The new position of the eyes suggest that larvae of this species will now have binocular vision in the antero-lateral projecting visual field, and an active pursuit predator behaviour characteristic of other streamlined larvae (Pankhurst, 1994).

In conclusion, this study suggests that the early rod generation in lanternfish larvae may be related to the constraints associated with visual planktivory away from well-lit surface waters. On the other hand, although cones differentiated during the retina embryogenesis of these lanternfish larvae, their sequential loss and increase in rod OS lengths are interpreted as adaptive responses to an impending deep meso- and bathypelagic adult life. Changes in the location and orientation of eyes with respect to the head from pre-flexion to post-flexion stages and the progressive rounding of the eye shape were observed in Myctophinae species. These variations can be explained in terms of changes in visual ecology strategies in preparation to the deep habitat where light is less unidirectional. Therefore light could be an important physical factor that structures the vertical distribution of these species.

Acknowledgments

The work undertaken by AB at the James Cook University was supported by a Spanish Ministry of Science postdoctoral fellowship. PMP was supported by a James Cook University Aquaculture and Emerging Species Research Advancement Program grant. This work was supported by the European Union in the framework of the MAST Program (MAS-CT96-0051) and by a Spanish grant (REN 2002-01339/MAR). The samples for the transmission electron microscope were processed in the Unidad Técnica de Microscopía Electrónica of the Barcelona University. We thank two anonymous reviewers for their accurate and constructive criticisms on an earlier version of the manuscript.

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