Spectral Tuning in the Eyes of Deep-Sea Lanternfishes (Myctophidae): A Novel Sexually Dimorphic Intra-Ocular Filter

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Key Words
Yellow pigment · Retinal filter · Spectral tuning · Myctophids · Opsins · Microspectrophotometry · Bioluminescence · Sexual dimorphism

Abstract
Deep-sea fishes possess several adaptations to facilitate vision where light detection is pushed to its limit. Lanternfishes (Myctophidae), one of the world’s most abundant groups of mesopelagic fishes, possess a novel and unique visual specialisation, a sexually dimorphic photostable yellow pigmentation, constituting the first record of a visual sexual dimorphism in any non-primate vertebrate. The topographic distribution of the yellow pigmentation across the retina is species specific, varying in location, shape and size. Spectrophotometric analyses reveal that this new retinal specialisation differs between species in terms of composition and acts as a filter, absorbing maximally between 356 and 443 nm. Microspectrophotometry and molecular analyses indicate that the species containing this pigmentation also possess at least 2 spectrally distinct rod visual pigments as a result of a duplication of the \(Rh1\) opsin gene. After modelling the effect of the yellow pigmentation on photoreceptor spectral sensitivity, we suggest that this unique specialisation acts as a filter to enhance contrast, thereby improving the detection of bioluminescent emissions and possibly fluorescence in the extreme environment of the deep sea. The fact that this yellow pigmentation is species specific, sexually dimorphic and isolated within specific parts of the retina indicates an evolutionary pressure to visualise prey/predators/mates in a particular part of each species’ visual field.

Introduction
The evolution of visual sensitivity appears to be driven primarily by the spectral range and intensity of available light within a species’ visual environment. As a result, different visual pigments are found in the retina of different species, with spectral sensitivities broadly matching the surrounding light conditions [Lythgoe and Partridge, 1989]. Spectral adaptation to the photic environment is mainly achieved by two types of tuning mechanisms: variation in the number of spectral classes of photoreceptors through the loss or duplication of opsin genes, and variation in the type of chromophore within the photoreceptor outer segment [see Bowmaker and Hunt, 2006; Bowmaker, 2008; Davies et al., 2012, for review].
Due to the physical properties of water, aquatic animals can be found in a wide range of spectral environments, from clear blue oceanic water to turbid brown river water, and as a result possess different subsets of cone classes ranging from the presence of at least 1 representative of each of the 4 cone classes (Australian lungfish [Bailes et al., 2007]) at one extreme to the retention of just a single cone class (several species of sharks [Hart et al., 2011]) at the other, although it is not uncommon amongst deep-sea fishes to find that all 4 cone classes have been lost to leave a rod-only retina [Hunt et al., 2001]. In the open ocean, light conditions vary greatly with depth, decreasing in intensity and spectral range as the short and long wavelengths are attenuated. In the deep sea, only very low intensities of sunlight in the blue-green range remain below 200 m, creating a relatively dark environment accompanied by a multitude of bioluminescent emissions that are also mainly concentrated in the blue-green part of the visible light spectrum [Widder, 2002, 2010].

The eyes of deep-sea teleost fishes represent a good example of visual adaptation in spectral sensitivity. Most species have adapted to their dim-light environment by losing their cone photoreceptors (photopic vision) in favour of a single type of rod photoreceptor (scotopic vision) containing a single visual pigment encoded by the Rh1 rod opsin gene. While most terrestrial and shallow water vertebrates usually have an Rh1 visual pigment maximally absorbing around 500 nm, many deep-sea teleosts have shifted the spectral sensitivity of their rod pigment towards shorter wavelengths to spectrally match their ambient photic environment, i.e. maximal absorbance around 480 nm [Crescitelli, 1990; Douglas and Partridge, 1997; Douglas et al., 2003]. The tuning mechanism responsible for the spectral shift toward shorter wavelengths in the Rh1 gene has been identified at the molecular level and occurs as a result of different combinations of substitutions at 8 different amino acid sites depending on the species [Hunt et al., 2001].

Like most mesopelagic fishes, lanternfishes (Myctophidae) possess several visual adaptations for life in the mesopelagic zone that serve to increase the sensitivity of the eye and optimise photon capture [de Busserolles et al., 2013, 2014a, b]. Lanternfishes generally lack cones and possess a single rod photoreceptor class with a peak spectral absorption tuned to the blue-green region of the visible spectrum [Partridge et al., 1992; Douglas and Partridge, 1997; Turner et al., 2009]. However, the presence of 2 photoreceptor classes has been found in a few species [Hasegawa et al., 2008; Turner et al., 2009]. In this study, we describe a novel visual adaptation, a retinal yellow pigment associated with a rod gene duplication, that is unique among vertebrates and assists lanternfishes to tune their visual sensitivity.

Material and Methods

Samples

Samples were collected in the Coral Sea during 3 cruises on the RV Cape Ferguson (AIMS, Townsville, Australia) in Autumn-Winter 2010–2012 under the following collection permits: Coral Sea waters (CSCZ-SR-20091001-01), Commonwealth waters (AU-COM2009051), GBRMPA (G09/32237.1) and Queensland Fisheries (133805) (Marshall, AEC#SNG/080/09/ARC), and in the Peru-Chile trench on the FS Sonne (Germany) in September 2010 (sampling permits obtained by the Chief Scientist, University of Tübingen, Germany). Sampling was performed at the surface (<5 m) during the night using an Isaacs-Kidd Midwater Trawl fitted with buoys or a neuston net. For each individual, the standard length and rostro-caudal eye diameter were measured with digital callipers (to a precision of 0.1 mm) prior to dissection and fixation (table 1). Following the guidelines of the NH&MRC Australian Code of Practice, under our University of Western Australia Animal Ethic Protocol (RA/3/100/917), eyes were then enucleated, the cornea and lens were dissected free from the eye cup and tissue was fixed specifically for different analyses (4% paraformaldehyde, RNALater, 100% alcohol, liquid nitrogen). One individual of Myctophum brachygnathum, sampled in Hawaii in May 2011 and fixed in 5% formalin, was provided by the American Museum of Natural History, New York, N.Y., USA (table 1).

Retinal Wholemounts and Spectrophotometry

For each species, eyes preserved in 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4, PFA) were dissected to reveal the location and shape of the yellow-pigmented patches of retinal tissue. Retinal wholemounts were made according to standard protocols [Stone, 1981; Coimbra et al., 2006, Ullmann et al., 2011] and mounted photoreceptor side up in 0.1 M phosphate buffer (pH 7.4) between two No. 1 glass coverslips. For one specimen of Symbolophorus rafinus, the eye was dissected on board ship directly after capture and the retina wholenuumped fresh in between two No. 1 coverslips and kept at ~20°C in a light-tight container until analysis.

The transmission of light (300–800 nm) through each retina was measured systematically every 0.5 or 1 mm (depending of the size of the retina) across the wholemount using spectrophotometry. Each transmission spectrum was ultimately converted to a corrected absorbance spectra at the wavelength of maximum absorbance of the yellow pigment (λYPmax). The detailed methods used to carry out the spectrophotometry analyses are provided in the supplementary material (for all online suppl. material, see www.karger.com/doi/10.1159/000371652). The λYPmax values were then mapped onto an outline of the retinal wholemount traced from the calibrated digital image in Adobe Illustrator CS4. The whole file was saved as a scalable vector graphics (.svg) file and imported into R v.2.15.0 (R Foundation for Statistical Computing, 2012) to construct the maps using custom scripts [Garza Gisholt et al., 2014]. For this study, the Gaussian kernel smoother from the Spatstat package was used to construct the iso-density maps [Baddeley and Marshall/Clarke/Hahne/Collin.](http://www.karger.com/doi/10.1159/000371652)
Cryosections

The retina of one individual of *Gonichthys tenuiculus*, preserved in 4% PFA, was processed for cryosectioning in order to visualise the position of the yellow pigmentation within the retina. Sections (40 μm in thickness) were cut using a Leica CM1900 cryostat-microtome and mounted in VectaMount Aqueous medium (Vector Laboratories, USA). The sections were observed under an Olympus BX50 compound light microscope and pictures were taken with an Olympus DP70 digital camera.

Identification of the Yellow Pigmentation

Yellow pigments have previously been observed in the eyes of vertebrates, either as a diffuse pigment within the lens and cornea or within the inner segment of the photoreceptors, i.e. in oil droplets or ellipsoids [Muntz, 1976; Appleby and Muntz, 1979; Goldsmith et al., 1984; Collin et al., 2003; Siebeck et al., 2003; Bailes et al., 2006]. These pigments have been identified as tryptophan derivatives [van Heyningen, 1971a, b; Thorpe et al., 1992] or mycosporine-like amino acids [Thorpe et al., 1993] in the lens, and as carotenoids [Goldsmith et al., 1984] in the retina. Extraction methods for the yellow pigment found in the retina of the lanternfishes followed therefore the classic extraction protocols for these various group of compounds, using appropriate standards. Retinai of *M. aurolaternatum* and *Hygophum proximum* were dissected while on board ship, deep-frozen in liquid nitrogen in small Eppendorf tubes and stored at –20 °C. In order to extract any water-soluble pigments (e.g. tryptophan derivatives and other amino acids), the retina of *M. aurolaternatum* was thawed at room temperature and homogenised several times in a glass tube with 1 ml of distilled water (3 min at 30 Hz) [Truscott et al., 1992; Thorpe et al., 1993]. Extraction of possible carotenoids was performed as described by Toomey and McGraw [2007, 2009]. The retina of *H. proximum* was thawed and 10 μl of trans-β-apo-8-carotenal (1 ng/μl; Sigma Aldrich) and 50 μl of retinyl acetate (1 ng/μl; Sigma Aldrich) were added to each sample.

Table 1. Summary of the individuals analysed in this study that presented a yellow patch of retinal tissue

<table>
<thead>
<tr>
<th>Species</th>
<th>Sex</th>
<th>SL, mm</th>
<th>Eye ø, mm</th>
<th>Sampling location</th>
<th>Patches, n</th>
<th>Patch location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. brachygnathum</em></td>
<td>F</td>
<td>65.7</td>
<td>6.8</td>
<td>Coral Sea</td>
<td>1</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>67.7</td>
<td>7.2</td>
<td>Coral Sea</td>
<td>1</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>69.7</td>
<td>7.5</td>
<td>Coral Sea</td>
<td>1</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>66.6</td>
<td>7.3</td>
<td>Coral Sea</td>
<td>1</td>
<td>T/V</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>67.7</td>
<td>7.5</td>
<td>Coral Sea</td>
<td>1</td>
<td>T/V</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>58.1</td>
<td>7.3</td>
<td>Hawaii</td>
<td>1</td>
<td>T/V</td>
</tr>
<tr>
<td><em>M. nitidulum</em></td>
<td>F</td>
<td>85.4</td>
<td>7.1</td>
<td>Peru-Chile Trench</td>
<td>1</td>
<td>T/C</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>80.0</td>
<td>6.9</td>
<td>Peru-Chile Trench</td>
<td>1</td>
<td>T/C</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>77.9</td>
<td>6.9</td>
<td>Peru-Chile Trench</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td><em>M. obtusirostre</em></td>
<td>M</td>
<td>92.4</td>
<td>10.6</td>
<td>Coral Sea</td>
<td>1</td>
<td>V</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>90.9</td>
<td>10.6</td>
<td>Coral Sea</td>
<td>1</td>
<td>V</td>
</tr>
<tr>
<td><em>M. lychnobium</em></td>
<td>F</td>
<td>106.1</td>
<td>11.1</td>
<td>Coral Sea</td>
<td>2</td>
<td>D + C/V</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>83.3</td>
<td>9.4</td>
<td>Coral Sea</td>
<td>2</td>
<td>D + C/V</td>
</tr>
<tr>
<td><em>M. spinosum</em></td>
<td>F</td>
<td>87.5</td>
<td>8.7</td>
<td>Coral Sea</td>
<td>2</td>
<td>D + C/V</td>
</tr>
<tr>
<td><em>M. aurolaternatum</em></td>
<td>J</td>
<td>57.8</td>
<td>5.3</td>
<td>Coral Sea</td>
<td>1</td>
<td>V</td>
</tr>
<tr>
<td><em>S. rufinus</em></td>
<td>J</td>
<td>73.7</td>
<td>6.6</td>
<td>Coral Sea</td>
<td>1</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>62.5</td>
<td>6.1</td>
<td>Coral Sea</td>
<td>1</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>31.6</td>
<td>2.8</td>
<td>Coral Sea</td>
<td>1</td>
<td>D</td>
</tr>
<tr>
<td><em>S. evermanni</em></td>
<td>J</td>
<td>65.8</td>
<td>6.7</td>
<td>Coral Sea</td>
<td>1</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>53.8</td>
<td>4.8</td>
<td>Coral Sea</td>
<td>1</td>
<td>D</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>48.1</td>
<td>4.5</td>
<td>Coral Sea</td>
<td>1</td>
<td>D</td>
</tr>
<tr>
<td><em>G. tenuiculus</em></td>
<td>M</td>
<td>49.4</td>
<td>3.5</td>
<td>Peru-Chile Trench</td>
<td>2</td>
<td>D + V</td>
</tr>
<tr>
<td></td>
<td>?</td>
<td>43.1</td>
<td>2.9</td>
<td>Peru-Chile Trench</td>
<td>2</td>
<td>D + V</td>
</tr>
<tr>
<td></td>
<td>?</td>
<td>40.6</td>
<td>2.7</td>
<td>Peru-Chile Trench</td>
<td>2</td>
<td>D + V</td>
</tr>
<tr>
<td><em>H. proximum</em></td>
<td>J</td>
<td>43.3</td>
<td>5.2</td>
<td>Coral Sea</td>
<td>1</td>
<td>D</td>
</tr>
</tbody>
</table>

For each individual, the sex (F = female, M = male, J = juvenile, ? = unknown), standard length (SL), eye diameter (ø), location of sampling and number of retinal yellow pigmented patches and their location (C = central, D = dorsal, T = temporal, N = nasal, V = ventral) are given.
added to the sample as internal standards. The sample was then homogenized in 1 ml of methanol for 3 min at 30 Hz and centrifuged (3 min at 13,000 rpm), and the supernatant was transferred into a glass tube. The procedure was repeated twice with 1 ml of 1:1 hexane:tert-butyl methyl ether (MTBE) and the supernatants combined. Since the yellow pigment was still present in the tissue and not in the supernatant, we repeated the procedure twice more with 1 ml of dimethyl sulfoxide (DMSO) [Sedmak et al., 1990].

Microspectrophotometry
The eyes of 3 species of lanternfishes, i.e. *M. obtusirostre*, *M. spinosum* and *S. evermanni*, were preserved for microspectrophotometry (MSP) according to Hart et al. [2011]. Specimens were collected alive and were dark adapted for 15 min before being euthanized following the guidelines of the NH&MRC Australian Code of Practice, under the University of Western Australia Animal Ethics Protocol (RA/3/100/917). Eyes were removed in the dark under dim red light, the cornea was removed and the whole eye was lightly fixed for 30 s in 2.5% glutaraldehyde in phosphate-buffered saline (PBS, 340 mosm · kg⁻¹, pH 7.0), washed in PBS for 30 s and stored in PBS at 4 °C in a light-tight container until they were analysed back in the laboratory 3–4 weeks later.

In all cases, small pieces (~1 × 1 mm to 3 × 3 mm) of retina were mounted between No. 1 glass coverslips in a drop of 4% agar (MW 282,000; Sigma D-7265). Absorbance spectra (330–800 nm) were made using a computer-controlled single-beam wavelength-scanning microspectrophotometer [Hart et al., 2004, 2011, 2012]. Due to the very small diameter of the rod photoreceptors of the lanternfish species analysed (<1 μm [de Busserolles et al., 2014b]), longitudinal ‘end-on’ absorbance spectra were measured from patches of rod outer segments. A sample scan was made by aligning the measuring beam (typical dimensions 10 × 10 μm) within a patch of rods in a specific part of the retina and recording the amount of light transmitted at each wavelength across the spectrum. Baseline scans were made in an identical way to sample scans but from a cell-free area of the preparation. The transmittance (ratio of sample to baseline signal) of the outer segments was calculated at each wavelength and converted to absorbance to give a pre-bleach spectrum. Each patch of rods was then bleached with broadband white light for 2 min and subsequent sample and baseline scans were made to create a post-bleach absorbance spectrum. Because we were measuring the absorbance through a patch of retina that contains other absorbing material (i.e. other retinal layers, yellow pigment) and not through a single rod outer segment, a difference spectrum was calculated by subtracting the post-bleach scan from the pre-bleach scan. Only spectra that satisfied established selection criteria [Levine and MacNichol Jr, 1985; Hart et al., 1998] were retained for further analysis. Difference absorbance spectra were analysed as described elsewhere [MacNichol Jr, 1986; Govardovskii et al., 2000; Hart, 2002] to provide an estimate of the wavelength of maximum absorbance (λmax) of the retinal pigment contained in the rods, assuming that only one type of rod was present within the patch analysed. The mean λmax of a given pigment was then calculated from these individual patch λmax values. For display purposes, a mean difference absorbance spectrum was calculated by averaging the acceptable individual absorbance spectra.

To investigate the possibility that the photoreceptors of some species contained a mixture of visual pigment molecules utilizing both the A1 and the A2 chromophores, mean pre-bleach absorbance spectra (smoothed with a variable point unweighted running average) were fitted iteratively using the Excel Solver function in a custom macro written by N.S.H. with mixed-chromophore pigment templates to find the combination of visual pigment λmax (pure A1) and A1/A2 ratio that gave the smallest sums of squares deviation between the real and modelled spectra between 0.8 and 0.2 normalised absorbance on the long-wavelength limb of the real spectrum (approximately the same region used to estimate λmax) [Temple et al., 2010]. We made the assumption that only a single type of visual pigment opsin protein was expressed in each rod within the patch of rods and used established A1 and A2 pigment templates [Govardovskii et al., 2000] and a known relationship between the λmax values of A1 and A2 visual pigment pairs [Parry and Bowmaker, 2000].

Molecular Analyses
Eye and muscle tissues from *S. evermanni* were preserved for molecular analyses by immersion in RNAlater and 100% alcohol, respectively, followed by storage at −20°C. Genomic DNA (gDNA) was extracted from the muscle tissue using a DNeasy Blood and Tissue Kit (Qiagen) following the protocol provided by the manufacturer. First-round PCR amplifications utilized the ‘All-Opsins’, ‘All-Species’ (AOAS) degenerate PCR primer set F1/R2 designed by Davies et al. [2009]. A second semi-nested PCR was carried out with primer sets F1/R1 and F2/R2 [Davies et al., 2009]. The sequences of the primers used in this study along with the annealing temperatures used and expected PCR products are provided in online supplementary table S1.

RNA was extracted from eye tissue using a PureLink RNA Mini Kit (Invitrogen) and cDNA synthesized using a 5′/3′ RACE Kit, 2nd generation (Roche), according to the manufacturer’s instructions. Ompin sequences were amplified from cDNA using primer sets designed to amplify vertebrate opsin proteins. The resulting amplified fragments were sequenced and the sequences used to design primers for RACE in order to extend the sequences at the 3′ ends. All PCR amplifications were performed using a HotStarTaq DNA Polymerase Kit (Qiagen). Following a heat activation step of 15 min at 95°C, 45 cycles were performed with a denaturation step of 30 s at 94°C, an annealing step of 60 s at 45–50°C, an extension step of 90 s at 72°C, and a final extension of 10 min at 72°C. Gel-purified (Wizard SV Gel and PCR Clean-Up System; Promega) PCR products were sequenced via the Sanger method.

Phylogenetic trees, including the *S. evermanni* opsin genes sequenced in this study, were constructed by neighbour joining [Saitou and Nei, 1987] and maximum likelihood using nucleotide sequences. All sequences were aligned in Clustal Omega [Sievers et al., 2011] and refined by eye. The parameters for the phylogenetic analysis, carried out using the MEGA phylogenetic package [Tamura et al., 2013], were pair-wise deletion and Kimura 2-parameter correction. The degree of support for internal branching was assessed by bootstrapping with 1,000 replicates.

Modelling of the Association of the Yellow Pigment and the Visual Pigments
The effect of the yellow pigmentation on the spectral sensitivity of the rods was modelled for 2 species: *M. obtusirostre* and *S. evermanni*. For each species, the quantal spectral sensitivity of the rod photoreceptors associated with the yellow pigmentation was modelled by multiplying the species-specific axial spectral absorbance of the rod outer segment by the spectral transmittance of the
species-specific overlying yellow pigmentation. We used visual pigment templates [Govardovskii et al., 2000] of appropriate $\lambda_{\text{max}}$ for the modelling. Normalised absorbance templates were converted to axial outer segment absorbance by assuming a visual pigment specific (decadic) absorbance of 0.013 μm$^{-1}$ (rhodopsin [Turner et al., 2009]) and a rod outer segment length of 39 μm for S. evermanni [de Bussyel et al., 2014b] and 45 μm for M. obtusirostre as per a similar species (M. brachygnathum [de Bussyel et al., 2014b]). Measurements of ocular media transmittance could not be made from fresh specimens at sea and so were excluded from the modelling.

Results

Description of the Yellow Pigment

Yellow pigmentation within the retina was noted in 10 species of lanternfishes from 4 different genera all belonging to a single subfamily, i.e. the Myctophinae (table 1). After detailed observation of 6 species, the distribution of the yellow pigmentation appeared to be species specific, varying in location, shape, size and number of patches (fig. 1a–f). Maps of the corrected absorbance at $\lambda_{\text{YPmax}}$ for each species correlated very well with the visual observations and indicated that the pigmentation was restricted to well-defined retinal regions (fig. 1g–l). The maximum corrected absorbance at $\lambda_{\text{YPmax}}$ varied between species from 0.78 (S. evermanni, fig. 1j) to 2.02 (G. tenuiculus, fig. 1g). Two of the 6 species analysed showed 2 patches of yellow pigmentation; G. tenuiculus possesses 2 ovoid-like patches, i.e. a small one in the dorsal part of the retina and a larger one in the ventral retina (fig. 1a), whereas M. lychnobotium possesses extensive pigment patches covering the entire dorsal part of the retina and the centro-ventral retina arranged in a streak-like pattern (fig. 1e). The 4 other species all possess a single patch of yellow pigmentation. H. proximum, S. rufinus and S. evermanni all have an ovoid-shaped patch situated in the dorsal or dorso-nasal part of the retina (fig. 1b–d, respectively), whereas M. obtusirostre possesses a streak-like patch in the temporo-ventral area (fig. 1f). The density of the yellow pigmentation appears to vary between species, with some showing very intense yellow pigmentation (i.e. G. tenuiculus, H. proximum, S. rufinus and S. evermanni, fig. 1a–d) and others paler pigmentation (i.e. M. lychnobotium and M. obtusirostre, fig. 1e, f).

Sections through the retina of G. tenuiculus reveal that the yellow pigmentation is situated within the outer nuclear layer (fig. 2). The pigmentation appears to be evenly distributed and does not appear to be compartmentalised within any organelles (i.e. oil droplets and ellipsosomes).

Sexual Dimorphism

A sexual dimorphism in the size, shape and location of the yellow pigmentation was observed in 2 species, i.e. M. brachygnathum and M. nitidulum, out of the 10 analysed (fig. 3). In M. brachygnathum (fig. 3a), this sexual dimorphism was observed in all 3 females and all 3 males examined. The females possess a small yellow patch in the central part of the retina just ventral to the optic nerve head. The males possess a larger yellow patch at the periphery of the temporo-ventral retina. In M. nitidulum (fig. 3b), there also appears to be a sexual dimorphism although this will have to be confirmed as only 2 females and 1 male were examined. In the females, the yellow patch takes the shape of a band extending from the temporoventral to the ventral retina, located ventral to the optic nerve head. In the male, the patch is approximately circular and located in the nasal periphery. At this stage, it is unknown whether the other 8 species with retinal yellow pigmentation also have a sexual dimorphism as either both sexes were not collected or only immature specimens were available (table 1).

Spectrophotometry and Pigment Extraction

Spectrophotometric analysis of the unfixed frozen retinal wholemount of S. rufinus revealed that the chemical fixation process had little effect on the absorbance of the yellow pigmentation, with a difference in $\lambda_{\text{YPmax}}$ between the fresh and fixed samples of only 3 nm and a difference in full width at half maximum (FWHM) of 12 nm (online suppl. fig. S1).

Spectrophotometric analysis of the retinal wholemounts of different species revealed that the yellow pigmentation acts as a short-wavelength-absorbing (long-pass) filter (fig. 4). The maximum absorbance of the yellow pigmentation ($\lambda_{\text{YPmax}}$) varies from 356 nm (M. obtusirostre, fig. 4f) to 443 nm (G. tenuiculus, fig. 4a). Comparison of the spectral curves, based on the $\lambda_{\text{YPmax}}$ from the different species (fig. 4), suggests that at least 3 different types of yellow pigments/filters are present with a peak absorbance at approximately 350, 380 and 440 nm. In addition, some of these pigments may be present as mixtures. This might be the case for H. proximum (fig. 4b), which seems to possess a mixture of the pigment present in M. obtusirostre (fig. 4f), G. tenuiculus (fig. 4a) and M. lychnobotium (fig. 4e).

In order to identify the nature of the yellow pigmentation, extraction was attempted from the retinas of 2 species, i.e. M. aurolaternatum and H. proximum, using water in the former species to extract water-soluble compounds and organic solvents in the latter species to extract carotenoids. However, in each case, the yellow pigment
Fig. 1. Diversity in the yellow pigmentation distribution across the retina of 6 species of lanternfishes. a–f Retinal wholemount visual observations. g–l Maps of corrected absorbance at the wavelength of peak absorbance of the yellow pigmentation $\lambda_{YP_{\text{max}}}$, G. tenuiculus (a, g), H. proximum (b, h), S. rufinus (c, i), S. evermanni (d, j), M. lychnobium (e, k) and M. obtusirostre (f, l). The arrows indicate the orientation of the retinal wholemount (T = temporal, V = ventral). Scale bars = 1 mm. Colour refers to the online version only.
proved to be refractory to extraction, thereby eliminating amino acids, tryptophan derivatives, lipophilic/hydrophilic pigments and carotenoids as the main constituents in the species analysed.

**Microspectrophotometry**

Microspectrophotometric results for the 3 species analysed are summarized in table 2. The mean difference spectra for each type of rod in each species are shown in figure 5. Except for *M. spinosum*, in which only a single spectral class of rod photoreceptor was identified (492 nm, fig. 5c), several spectral classes of rod photoreceptors were found in the other 2 species.

For *M. obtusirostre*, 2 classes of rod photoreceptors were identified with mean $\lambda_{\text{max}}$ values of 473 nm in the non-yellow retinal area and 527 nm in the area containing the yellow pigmentation (fig. 5b). On the basis of goodness of fit to established A$_1$ and A$_2$ visual pigment templates [Govardovskii et al., 2000], the pigment in the 473-nm rod was considered to represent 100% A$_1$ and the pigment in the 527-nm rod was considered to represent 100% A$_2$. However, given the known relationship between the $\lambda_{\text{max}}$ values of A$_1$ and A$_2$ visual pigment pairs [Parry and Bowmaker, 2000], it is very likely that these two pigments originate from two different opsins, since replacement of an A$_1$ by an A$_2$ chromophore would only shift the $\lambda_{\text{max}}$ of the 473-nm pigment to 484 nm.

For *S. severmanni*, 3 classes of rod photoreceptor were identified with mean $\lambda_{\text{max}}$ values of 476, 503 and 512 nm.
(fig. 5c). While the 476- and 503-nm pigments were found to be best fitted by $A_1$-based templates, the 512-nm pigment was best fitted by a template derived from a mixture of 47% $A_1$ and 53% $A_2$. Although both the 476- and the 503-nm pigments possess 100% $A_1$ chromophore, thereby indicating the presence of 2 different opsins, it is unlikely that the 512-nm pigment originates from a third opsin. Most probably, the 512-nm pigment contains the same opsin as the 503-nm pigment, with the difference in wavelength arising from the presence of both $A_1$ and $A_2$ chromophores within their outer segments.

Fig. 5. Mean bleaching difference absorbance spectra of the rod visual pigments in 3 species of lanternfishes, i.e. *S. evermanni* (a), *M. obtusirostre* (b) and *M. spinosum* (c). The wavelength of maximum absorbance ($\lambda_{\text{max}}$) is also provided for each species. Spectra (black lines) are fitted with visual pigment templates (grey lines [Govardovskii et al., 2000]) of appropriate $\lambda_{\text{max}}$ value. For *M. obtusirostre* (b), the absorbance spectra were measured in non-yellow- and yellow-pigmented areas of retinal tissue.

| Table 2. Spectral absorption characteristics of rod visual pigments in the retina of 3 myctophid species measured using MSP |
|-----------------------------|-----------------------------|-----------------------------|
|                            | *M. spinosum*               | *M. obtusirostre*           | *S. evermanni*               |
|                            | 1                           | 1                           | 2                           | 1       | 2  | 3       |
| Scans analysed, n          | 7                           | 4                           | 9                           | 4       | 10 | 6       |
| Chromophore                | $A_1$                       | $A_1$                       | $A_2$                       | $A_1$   | $A_1$ | $A_1 + A_2$ |
| Mean $\lambda_{\text{max}}$ (±1 SD), nm | 492.1 ± 4.3                 | 473.4 ± 1.7                 | 527.3 ± 1.1                 | 475.9 ± 1.2 | 503.4 ± 1.4 | 512.7 ± 7.4 |
| $\lambda_{\text{max}}$ of the mean spectrum, nm | 492.4                       | 473.2                       | 526.5                       | 476.0   | 503.5 | 511.9   |
| Maximum corrected absorbance | 0.020                      | 0.058                      | 0.055                      | 0.023   | 0.126 | 0.062   |
| Running average$^2$ $\lambda_{\text{max}}$ | 494                        | 477                        | 528                        | 477     | 505  | 510     |

$^1$ Measured at the $\lambda_{\text{max}}$ of the bleaching difference spectrum. $^2$ Variable-point unweighted running average maximum of the data, which is a measure of the wavelength of peak absorbance of the pigment that is independent of any assumptions as to the type of chromophore ($A_1$ or $A_2$) present.
Molecular Analyses

Two opsin transcripts, identified as encoded by 2 Rh1 opsin genes, were PCR-amplified from S. evermanni retinal cDNA. The sequences were aligned with the Rh1 rod opsins and representatives of all 4 cone opsin coding sequences of the zebrafish Danio rerio using Clustal Omega [Sieviers et al., 2011]. These alignments were then used to generate neighbour-joining and maximum-likelihood phylogenetic trees (online suppl. fig. S2). In both cases, both S. evermanni sequences fall into the same clade as the zebrafish Rh1 sequences, thereby confirming that both are Rh1 opsin orthologues. The two sequences were designated as Rh1-A and Rh1-B.

Rh1-A and Rh1-B differ at specific sites that have previously been shown to be important for spectral tuning [Hunt et al., 2001] (online suppl. fig. S3); Rh1-B possesses Tyr rather than Phe at site 261 and Ala rather than Ser at site 292. These two substitutions in Rh1-B would be expected to result in a long-wavelength shift in the absorbance peak of the pigment compared to Rh1-A, and extrapolation from pigments in other species with identical substitutions would indicate a red shift of around 20–25 nm [Yokoyama et al., 1995; Hunt et al., 1996]. This is consistent with the different $\lambda_{\text{max}}$ values found using MSP for rod photoreceptors in this species.

Despite several attempts to complete the sequence of Rh1-A, the 5' end remained truncated; the missing portion includes 2 potential tuning sites (sites 83 and 122). However, since Rh1A shows a spectral sensitivity similar to that of the other myctophid rod opsins (~470 nm), which possess Asp83 and Gln122 [Yokoyama et al., 2008], as does Rh1-B (the long-wave-shifted rod), it is unlikely that either of these sites in Rh1-A is substituted.

Rh1-A and Rh1-B nucleotide sequences from S. evermanni were aligned with all of the myctophid Rh1 opsin sequences available in GenBank, plus the Rh1 opsin gene nucleotide sequences of the short-fin pearleye Scopelanarchus analis, the zebrafish D. rerio, the yellow river scaleless carp Gymnocypris ecklonii and the elephant shark Callorhinchus miliii. The Rh2 opsin gene nucleotide sequence of D. rerio served as an out group. Neighbour-joining and maximum-likelihood analyses were carried out on the aligned nucleotide sequences and the resultant trees (fig. 6) both grouped the S. evermanni sequences with those from Electrona antarctica and the two Benthosema species. The precise location of the S. evermanni differed, however, between the two methods, with neighbour-joining placing the S. evermanni Rh1-B sequences subsequent to the evolution of the Benthosema genus and the maximum-likelihood method placing the S. evermanni Rh1-B sequence on a separate branch with the B. pterotum sequence only. The relevant bootstrap values are all relatively low, reflecting the overall similarity of the myctophid Rh1 sequences. It would appear, therefore, that the Rh1 duplication in S. evermanni arose within the clade defined by 3 genera, i.e. Symbolophorus, Benthosema and Electrona, but its precise location cannot be further verified.

Modelling of the Effects of the Yellow Pigment on Photoreceptor Spectral Sensitivity

Following our observations and measurements using MSP in yellow- and non-yellow-pigmented areas of the retina of M. obtusirostre, we concluded that the yellow pigmentation was only associated with the long-wave-shifted rod visual pigment and, as a result, only modelled this scenario in both species. Species-specific data were used to model the filtering effect of the yellow pigment on the photoreceptor spectral sensitivity (i.e. species-specific rod outer segment spectral absorbance and species-specific yellow pigment spectral transmittance).

Results show that the yellow filter decreases the absolute sensitivity at the peak of the long-wave-shifted rod visual pigment, shifts the overall sensitivity peak slightly toward longer wavelengths, and narrows the spectral sensitivity function by absorbing strongly at short wavelengths (fig. 7, 8). In M. obtusirostre, the yellow pigment narrows the FWHM bandwidth of the long-wave-shifted visual pigment from 248 nm to 142 nm (fig. 7) and shifts the peak of maximum absorbance of the rods 6 nm toward longer wavelengths.

In contrast to M. obtusirostre, 2 long-wave-shifted rod visual pigments were found in S. evermanni (fig. 8a).

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with the yellow pigment (fig. 8b), or at 476, 513 and 512 nm (fig. 8c) or 476, 503 and 520 nm (fig. 8d) if only one or the other of the long-wave-shifted visual pigments is associated with the yellow pigment.

**Discussion**

**The Unique Yellow Pigment in the Retina of Myctophids**

In this study, we describe a novel visual specialisation, a photostable yellow pigmentation, present in the retina of several species of lanternfishes. Retinal photostable yellow pigmentation has previously been observed in a number of animals including species of lamprey [Collin et al., 2003] and lungfish [Bailes et al., 2006], the ornate dragon lizard [Barbour et al., 2002] and Amazonian cichlid fishes [Muntz, 1973]. However, in contrast to these other animals, the yellow pigmentation in lanternfishes is restricted to well-defined areas (patches) of the retina, which appear to be species specific in terms of number, size, location and pigment density. Moreover, the location of the yellow pigmentation throughout the outer nuclear layer of the retina of myctophids differs from what has been previously observed in other species, where it is found within the distal region of the inner segment and/or diffusely distributed within the myoid region of the photoreceptors [Barbour et al., 2002; Collin et al., 2003; Bailes et al., 2006].

Unfortunately, the composition of the yellow pigmentation in myctophids could not be identified in this study. Colouration in animals is primarily due to the presence of melanins and carotenoids, although other types of pigment have been shown to be responsible for

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**Fig. 6.** Origin of the Rh1 rod opsin gene duplication in *S. evermanni* within the Myctophidae. Phylogenetic trees were constructed via the neighbour-joining [Saitou and Nei, 1987] (a) and maximum likelihood (b) methods using Rh1 opsin gene nucleotide sequences of *Diaphus metopoclampus* (JN544536), *D. rafinesqui* (JN412587), *D. watasei* (JN231003), *Stenobrachius leucopsar* (EU407251), *Lampanyctus alatus* (JN412575), *C. warmingii* (JN412573), *Bolilichthys indicus* (JN412574), *Benthosema subrubrite* (JN412576), *B. pterotum* (JN231002), *E. antarctica* (AY141258), *S. analis* (EF517404), *D. rerio* (HM367063), *G. eckloni* (EU606010) and *C. milii* (EF565167). The Rh2 opsin gene nucleotide sequence of *D. rerio* (NM131253) was added as a supplementary out group. The bootstrap confidence values are shown for each branch. The scale bar is calibrated at 0.05 substitutions per site.
animal colouration, i.e. porphyrins, pterins, flavins, psittacofulvins [McGraw, 2006] and amino acids [Thorpe et al., 1993]. Specifically, yellow pigmentation in ocular tissues has been identified as carotenoids (in pufferfish cornea [Appleby and Munzt, 1979], in avian retinal oil droplets [Goldsmith et al., 1984] and in the human macula [Bone et al., 1985]), as tryptophan derivatives (in the lens of terrestrial vertebrates [van Heyningen, 1971a, b] and in the lenses of deep-sea fishes [Thorpe et al., 1992]) and as mycosporine-like amino acids (in the lenses of deep-sea fishes [Thorpe et al., 1993]). Results from our study seem to exclude all of these compounds plus lipophilic or hydrophilic pigments (e.g. porphyrins and psittacofulvins) since water and non-polar solvents were also unsuccessful. However, extraction was only performed in 2 species: *M. aurolaternatum*, for which no spectrum is available, and *H. proximum*. Since no carotenoids were extracted in *H. proximum*, the pigment present in *H. proximum* and the two other species with similar spectra, i.e. *M. lychnobium* and *M. obtusirostre*, may therefore not be carotenoid based, unless the bond occurs in a way that...
does not allow extraction with standard methods. The spectra obtained for the pigment in the remaining three species, i.e. *G. tenuiculus*, *S. evermanni* and *S. rufinus*, are, however, different, with 2 peaks that are broadly similar to spectra obtained from carotenoids [Goldsmith et al., 1984]. So, although it would seem unlikely that the pigmentation has a different basis between species, this cannot be ruled out. Further analyses will have to be conducted to confirm the presence/absence of these compounds in all species and investigate other possibilities.

Melanins are often implicated in animal colouration and provide the black pigmentation of the retinal pigment epithelium. They exist, however, in two distinct forms, i.e. brown to black eumelanin and yellow to red pheomelanin. Melanin is also known to be difficult to extract from tissues due to its heterogeneous nature and its association with proteins [Taft, 1949; Liu et al., 2003]. Therefore, the difficulty in extracting the lanternfish yellow pigment could be consistent with the presence of a melanin and its colour would indicate that it is composed of pheomelanin. Although pheomelanin has yet to be directly observed in fishes [Ito and Wakamatsu, 2003], the presence of agouti signalling proteins (ASIP), which control the production of pheomelanin, in several fishes, i.e. goldfish [Cerda-Reverter et al., 2005], zebrafish and several species of pufferfishes [Klovins and Schiöth, 2005], indicates that it may be present; it could therefore be one of the components of the yellow pigmentation in lanternfishes.

Another unique feature of the yellow pigmentation in lanternfishes is its sexual dimorphism, observed so far in 2 species of the genus *Myctophum*. Although sexual dimorphism in the visual system has already been observed in insects (houseflies [Franceschini et al., 1981; Zeil, 1983], march flies [Hornstein et al., 2000], moths [Meyer-Rochow and Lau, 2008] and butterflies [Arikawa et al., 2005; Sison-Mangus et al., 2006]), crustaceans (copepods [Land, 1984, 1988]) and primates [Hunt et al., 2005], to our knowledge, this is the first observation of sexual dimorphism in the visual system of any non-primate vertebrate. Within these examples, sexual dimorphism in the visual system has been observed at several levels, i.e. eye morphology (flies [Franceschini et al., 1981; Zeil, 1983]), retinal organisation (flies [Zeil, 1983; Hornstein et al., 2000]), and spectral sensitivity (butterflies [Arikawa et al., 2005; Sison-Mangus et al., 2006] and primates [Hunt et al., 2005]). Lanternfishes show a new type of sexual dimorphism in the visual system, functionally similar to the visual system of the small white butterfly *Pieris rapae crucivora* [Arikawa et al., 2005], where a filter is present in the ommatidia, which tunes visual sensitivity. However, in contrast to the small white butterfly, both male and female lanternfishes possess the filter but in different areas of the retina.

**Visual Pigments and Spectral Tuning in Myctophids**

The presence of different spectral classes of the rod photoreceptors in 2 lanternfish species with yellow retinal pigmentation indicate the presence of more than one visual pigment. While most lanternfish species possess a single photoreceptor class [Turner et al., 2009], 2 photoreceptor classes have been observed in 4 other species, i.e. *Ceratoscopelus warmingii* [Douglas et al., 2003], *H. proximum*, *M. aurolaternatum* [Turner et al., 2009] and *M. nitidulum* [Hasegawa et al., 2008], with the last 3 species all possessing yellow pigmentation (this study). Based on the conclusions of this study and known relationships between the λmax values of A1 and A2 visual pigment pairs [Parry and Bowmaker, 2000], the presence of two different opsins can be predicted for *H. proximum*, *M. nitidulum*, [Hasegawa et al., 2008; Turner et al., 2009], *M. obtusirostre* and *S. evermanni*. This was confirmed at the molecular level for *S. evermanni* (this study).

A pure rod retina with a single *Rh1* pigment is typical among deep-sea fishes [Hunt et al., 2001]. However, analysis of the rod opsin coding sequences expressed in *S. evermanni* revealed the presence of 2 *Rh1* opsin genes (*Rh1*-A and *Rh1*-B). This is only the second case of a rod opsin duplication recorded for a deep-sea fish, the first being the pearleye *S. analis* [Pointer et al., 2007]. However, in contrast to *S. analis*, for which the duplication of *Rh1* results in 2 spectrally similar visual pigments (486 and 479 nm [Pointer et al., 2007]), duplication in *S. evermanni* gives rise to 2 spectrally distinct visual pigments, with peaks at 476 and 503 nm. Moreover, while the 2 *Rh1* genes are phylogenetically different in *S. analis*, indicating an early duplication in evolutionary history [Pointer et al., 2007], the *Rh1*-A and *Rh1*-B genes in *S. evermanni* are phylogenetically very similar, indicating the origin of the duplication within a clade defined by 3 genera of myctophids. The fact that this duplication appeared at a specific stage in the evolutionary history of the family, and is only present in the Myctophinae subfamily, could explain why it has not been reported previously.

*Rh1* gene duplications have only been reported in teleost fishes and, even then, are quite rare. In addition to...
the two deep-sea species mentioned above, it has been observed in the cyprinid zebrafish *D. rerio* [Morrow et al., 2011] and a few eel species [Hunt et al., 2013]. The expression of the second *Rh1* gene in the eel is associated with a change in the spectral conditions of their habitat [Archer et al., 1995]. Myctophids show marked differences in habitat depending on their developmental stage, with larvae inhabiting the well-lit surface layers of the ocean [Sabates et al., 2003] and juvenile and adults occupying the deeper mesopelagic zone. Moreover, larvae and juvenile/adults also possess different types of photoreceptors, the larvae having both rods and cones [Bozzano et al., 2007], while juveniles/adults possess only rods. There is therefore a change in visual pigment expression between larval and juvenile/adult stage myctophids. However, microspectrophotometric results predict that both *Rh1* genes are expressed in the retina of the adult lanternfish, although it is unknown at this stage if both genes are also expressed in the larvae. While most deep-sea organisms produce bioluminescent emission in the blue-green region of the visible spectrum, several organisms do produce longer wavelength signals [Widder, 2010], the most extreme case being the red bioluminescence produced by some stomiid dragonfishes [Widder et al., 1984]. Some of these far-red illuminating fishes are known to prey on myctophids [Sutton and Hopkins, 1996], and the long-wave-shifted visual pigment in some of these lanternfishes (i.e. *M. nitidulum* and *M. obtusirostre*) could potentially have evolved as a mechanism to detect these predators [Hasegawa et al., 2008; Turner et al., 2009].

*Putative Functions of the Yellow Pigment in Myctophids*

Intra-ocular filters are thought to have many functions in vertebrates. In well-lit environments, they may improve visual acuity by reducing chromatic aberration and scatter (yellow lens and cornea [Walls, 1931; Walls and Judd, 1933; Muntz, 1973; Barbour et al., 2002]), enhance the discrimination of colours by narrowing the spectral sensitivity of the visual pigment (i.e. oil droplets [Hart, 2001]), filter out the potentially damaging short wavelengths present in bright environments [Collin et al., 2003] or allow the detection of fluorescent light emitted by certain organisms [Sparks et al., 2014]. In the deep sea, only 2 types of intra-ocular filters have been observed previously in teleosts: yellow lenses [Douglas and Thorpe, 1992] and the yellow pigmentation of the retina found in myctophids. In the mesopelagic zone, yellow lenses may increase hue discrimination in order to better visualise bioluminescent flashes or to break down the counterilluminating camouflage of other species [Muntz, 1976; Somiya, 1976, 1982; Douglas and Thorpe, 1992]. Indeed, during the day in the mesopelagic zone, the simultaneous presence of downwelling sunlight and bioluminescent flashes might render the detection of both signals difficult. More particularly, the presence of downwelling sunlight might reduce the contrast, and thereby the visibility, of bioluminescent emissions, which could be detrimental in terms of survival. However, since most bioluminescent emissions have a broader spectrum than downwelling light [Herring, 1983], yellow lenses might prevent this problem by cutting off most of the background illumination, thereby accentuating the signal. Lanternfish species with a yellow retinal pigmentation are found at very different depths during the day [de Busserolles et al., 2013]. However, at night they all migrate towards the surface to depths of <4 m [de Busserolles et al., 2013], where downwelling light produced by the moon and stars [Johnsen et al., 2004] may reduce the visibility of bioluminescent emissions. Therefore, in a similar way to yellow lenses, the presence of retinal yellow pigmentation could enhance the contrast of bioluminescent emissions against the night downwelling light in specific regions of the visual field.

Another potential function or outcome of yellow filtering in the mesopelagic realm is to increase the contrast of fluorescent objects such as siphonophores and other gelatinous zooplankton, which constitute regular prey [Kinzer and Schulz, 1985; Hopkins and Gartner, 1992; Beamish et al., 1999] and potential predators [Haddock et al., 2005; Pages and Madin, 2010] of myctophids. Three areas of evidence support this idea. Firstly, several studies have shown that a number of mesopelagic animals both bioluminesce and fluoresce [Haddock et al., 2005, 2010; Widder, 2010]. Secondly, the light distribution in the mesopelagic zone, peaking around 475 nm (downwelling light and bioluminescence [Widder, 2010]), is well matched to the excitation wavelengths needed to trigger a variety of fluorescent marine compounds [Mazel et al., 2004; Haddock et al., 2005; Alieva et al., 2008; Vogt et al., 2008] and this means that, at the right depth and resulting light level, fluorescence may be a significant component of the signals from such animals. Thirdly, as the new wave of photographers interested in capturing fluorescence are aware [Mazel, 2005; www.nightsea.com], a yellow blocking filter enhances the contrast at longer wavelengths for yellow, orange or red emissions of fluorescence [Sparks et al., 2014]. This is because yellow removes the wash of blue excitation and leaves only the glowing fluorescent emission component, a highly contrasting signal, especially in...
dim illumination. As a result, in addition to possibly enhancing the bioluminescent contrast, the yellow filters in the eyes of myctophids may also enable them to see fluorescent gelatinous prey and/or predators and any other fluorescent signals at depth.

Different species of lanternfishes possess different yellow-pigmented areas in the retina that may serve to enhance sensitivity to bioluminescence and/or fluorescence in different parts of the visual field. This implies inter-specific differences in behaviour. Myctophids show a variety of inter-specific differences in behaviour with respect to their depth distribution [Karnella, 1987], migration pattern [Watanabe et al., 1999] and diet [Kozlov, 1995; Shreeve et al., 2009; Van Noord et al., 2013]. Since all the species which possess a retinal yellow pigmentation vertically migrate to the surface at night, inter-specific differences in the location of the yellow pigmentation could potentially reflect differences in diet, a consequence of different nutritional requirements, niche partitioning and/or body size differences between males and females [Shine, 1989]. Unfortunately, data on diet are quite limited for lanternfishes and highly variable between seasons and geographic regions [Kozlov, 1995], making any diet/vision comparison impossible at this stage. Moreover, no studies have been conducted on possible diet differences between sexes, which would potentially explain the observed sexual dimorphism in the retinal yellow pigmentation. Sexual dimorphism in food choices has been reported in a number of organisms, as a consequence of different nutritional requirements, food competition, habitat and body size between males and females [Shine, 1989]. Sexual dimorphism in size has been observed in several lanternfish species [Gartner, 1993; Braga et al., 2008; Flynn and Paxton, 2012] and some differences in distribution between sexes have been suggested [Flynn and Paxton, 2012], supporting the possible role of the retinal yellow pigmentation in food choice.

The sexual dimorphism in the retinal yellow pigments implies a possible role in intra-specific/sexual communication. All of the species possessing the yellow pigment are also sexually dimorphic in the location and size of their caudal luminous organs, with all males possessing a large supracaudal organ and all females possessing a smaller infracaudal organ [Herring, 2007]. If the retinal yellow pigmentation is involved in sexual communication, inter-specific differences in the shape, size and location of the retinal pigment patches could be associated with differences in luminous organ location, size and shape between species, taking into consideration the position of the fish in the water column. Unfortunately, this information is not available for the species analysed and it is unknown how males and females position themselves with respect to one another in the water column. Further analyses of the retinal yellow pigmentation in both sexes, at several different life stages and from all the species possessing this yellow pigment will shed more light on the function and development of this new visual specialisation.

Overall, our findings indicate an evolutionary pressure to visualise prey/predators/mates in a specific part of each species’ visual field. Our results also add a new dimension to the contribution made by recent studies [Davis et al., 2014; Kenaley et al., 2014] on the evolutionary history in the deep-sea by identifying a visual ‘arms race’, in term of visual adaptation, between lanternfishes and dragonfishes. In fact, while dragonfishes have evolved a secret communication/predation channel by using red bioluminescence, lanternfishes, by virtue of their visual sexual dimorphism, are taking the race a step further by inviting ‘sex in the blue-light district of the deep sea’. It appears we have yet to unlock all of the secrets of bioluminescence communication in the deep sea.

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References


