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Polarization sensitivity and retinal topography of the striped pyjama squid (*Sepioloidea lineolata* – Quoy/Gaimard 1832)

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SUMMARY

Coleoid cephalopods (octopus, cuttlefish and squid) potentially possess polarization sensitivity (PS) based on photoreceptor structure, but this idea has rarely been tested behaviourally. Here, we use a polarized, striped optokinetic stimulus to demonstrate PS in the striped pyjama squid, *Sepioloidea lineolata*. This species displayed strong, consistent optokinetic nystagmic eye movements in response to a drum with stripes producing *e*-vectors set to 0 deg, 45 deg, 90 deg and 135 deg that would only be visible to an animal with PS. This is the first behavioural demonstration of a polarized optokinetic response in any species of cephalopod. This species, which typically sits beneath the substrate surface looking upwards for potential predators and prey, possesses a dorsally shifted horizontal pupil slit. Accordingly, it was found to possess a horizontal strip of high-density photoreceptors shifted ventrally in the retina, suggesting modifications such as a change in sensitivity or resolution to the dorsal visual field.

Key words: *Sepioloidea lineolata*, optokinetic response, e-vector, nystagmus, polarization sensitivity.

INTRODUCTION

Coleoid cephalopods (octopus, cuttlefish and squid) possess eyes similar in structure to vertebrate eyes (Muntz, 1999). Although most species are thought to be colour-blind, the structure of their photoreceptors suggest polarization sensitivity (PS) – a feature they might exploit in their marine environment (Waterman, 1988; Shashar et al., 1996; Shashar et al., 1998; Shashar et al., 2000; Shashar et al., 2001b; Wehner, 2001; Boal et al., 2004; Mäthger and Hanlon, 2006). Here, we demonstrate PS in *S. lineolata* for the first time using a modified optokinetic apparatus and discuss how it might play a role in their visual ecology.

Sepioloidea lineolata

The striped pyjama squid, *Sepioloidea lineolata* (Quoy/Gaimard 1832), is a small species belonging to the Sepiadariidae family and reaching up to 70 mm in mantle length (Norman, 2000). They are typically found buried in the fine substrates of mudflats and seagrass beds by day at a depth of up to 20 m, emerging at night to feed on small fish and crustaceans. An unusual feature of this species is the dorsal position of the pupil – a likely result of the way this animal buries itself in the substrate and hunts from its hidden position. In addition to the dorsal pupil, we also show that pyjama squid possess a ventrally shifted band of high-density photoreceptors, indicating increased acuity in their dorsal visual field (Fig. 1A–C).

S. lineolata spend much of their time buried in the substrate, using their arms to flick particles over their body, completely concealing themselves. Only the tops of their eyes are left exposed, explaining the dorsal-shifting of the pupil. Being embedded in the ocean floor also means that eye movements, rather than whole-body movements, are more appropriate for responding to visual stimuli without the risk of breaking crypsis.

Polarized light and polarization sensitivity

Light can become polarized by reflection, refraction and scattering by non-metallic objects (Nilsson and Warrant, 1999; Cronin and Shashar, 2001; Wehner, 2001) such as some air and water molecules and suspended particles, the surface of the ocean, the scales of silvery fish and the waxy cuticles of arthropods (Marshall et al., 1999; Cronin and Shashar, 2001). As this occurs, photons can become orientated, or polarized, into a single vibrational plane. The resulting angle of this vibrational plane is referred to as the electric vector (e-vector) of polarization.

PS in coleoid cephalopods results from the possession of orthogonally arranged microvilli on the photoreceptors of the retina (Saibil, 1982; Hanlon and Messenger, 1996; Cronin and Shashar, 2001; Shashar et al., 2001b). The outer segments of these photoreceptors possess sets of stacked microvilli on opposite sides called rhabdomeres. The rhabdomeres of single photoreceptors are orthogonal to the rhabdomeres on adjacent photoreceptors. This results in a square, lattice-like arrangement of cells (Fig. 2). Visual pigment molecules lie within the microvillar tubular membranes, and the long axes of these molecules are thought to be aligned parallel to the long axes of the microvilli in which they are positioned. This results in highest sensitivity to e-vectors vibrating on axes parallel to those of the microvilli, and therefore, through a combination of membrane anatomy and molecular alignment, two PS directions result: horizontal and vertical (Moody and Parriss, 1961; Snyder, 1973; Saibil, 1982; Saidel et al., 1983; Nilsson and Warrant, 1999; Shashar et al., 2001b).

A potentially significant use for PS in the animal kingdom, particularly in the marine environment, is enhancing the levels of contrast in the visual field – this can be achieved through a variety of mechanisms. PS might decrease the deleterious effects of scattered light that falls between an animal and an object, and

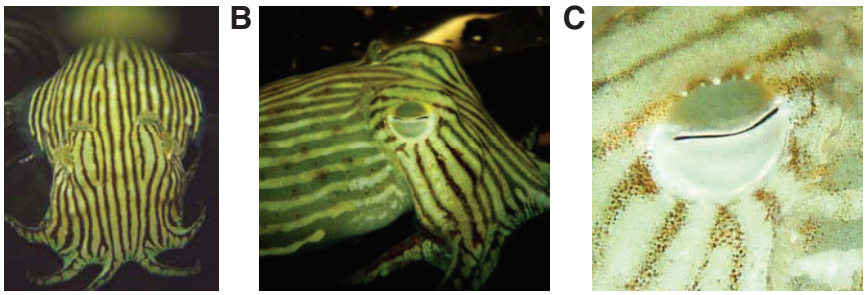

Fig. 1. (A,B) *S. lineolata* showing its striking, striped pattern and large, protruding eyes. (C) The dorsally shifted pupil slit.

between that object and its surroundings (Cronin and Shashar, 2001; Horvath and Varju, 2004; Shashar et al., 2001b; Wehner, 2001), and we predict it might also reduce the confounding effects of flicker in shallow water. If the object reflects or refracts light that is polarized differently to that of its surroundings, it might also appear more conspicuous against its background when viewed by a polarization-sensitive visual system.

In addition to contrast enhancement, some cephalopods and crustaceans might use polarized patterns generated on parts of their body, such as the cuticle or skin, for intra-specific communication. For example, the stacked protein-layer structure of the iridophores in the skin of the arms and head of some cephalopods is capable of reflecting ambient light, resulting in polarization (Cronin and Shashar, 2001; Shashar et al., 2001a; Cronin et al., 2003). It has also been shown that these polarized signals can pass through overlying, expanded chromatophores unchanged, which would enable these animals to communicate with conspecifics while maintaining a particular body pattern, such as a camouflage pattern, which can be highly pigmented (Mäthger and Hanlon, 2006).

Eye movements

Optokinesis, or the optokinetic response (OKR), is an innate tendency for the eyes to follow large-scale moving stimuli within the visual field until they can no longer turn in the same direction any further. The eyes then perform a fast, anti-compensatory movement, or flick, in the opposite direction to that of the stimulus and repeat the process for as long as the movement occurs (Walls, 1962; Carpenter, 1988; Fritsches and Marshall, 2002). This repetitive, compensatory slow-phase movement in the same direction as that of the stimulus, and anti-compensatory fast-phase movement in the opposite direction, is known as nystagmus – its function is to stabilize and maintain an image on the retina so there is no, or minimal, blur encountered as the visual field moves (Walls, 1962; Horridge and Sanderman, 1964; Carpenter, 1988; Fritsches and Marshall, 2002). OKRs can be observed in stationary subjects (such as *S. lineolata*), or subjects whose movement has been restricted experimentally.

Visual responses to moving, large-field stimuli, such as black-and-white vertical stripes, are known in the cuttlefish *Sepia officinalis* (Collewijn, 1970; Messenger, 1970; Groeger et al., 2005). In this study, we replaced the black-and-white drum with a drum made of a linear polarizing filter producing alternating e-vectors. These alternating e-vectors, when viewed through a piece of linear polarizing filter or a polarization-sensitive visual system, appear as stripes with different levels of contrast (like the contrast between black-and-white stripes). Thus, similar responses between a black-and-white drum and a polarizing drum are indicative of a positive behavioural response to the polarizing stimulus – that is, the animal perceives polarizing contrast.

MATERIALS AND METHODS

Two *Sepioloidea lineolata* were caught using sein nets in seagrass beds and mudflats at Dunwich, North Stradbroke Island, Queensland, Australia, in April 2008. The squid, approximately 25–30 mm in mantle length, were kept in tanks at Moreton Bay Research Station with a flow-through filtration system. The squid were fed live shrimp and small crabs. All experiments were conducted in accordance with: the University of Queensland Animal Ethics Committee, permit number SBS/738/08/ARC; Moreton Bay Marine Parks Regulation Permit, permit number QS2008/CVL625; and Queensland Government Department of Primary Industries and Fisheries, permit number 55604. Behavioural experiments were conducted first, and the same animals were then used for the anatomical analysis of the retina.

Retinal topography

Following behavioural experimentation, retinae were removed from fixed specimens and bleached in a solution of 6% H_2O_2 in 425 mOsm phosphate buffer solution (PBS), which was made up to a pH of 11.94 using 1 mol l^{-1} KOH. This protocol was adapted from that used on fish in a study by Kröger and Wagner (Kröger and Wagner, 1998).

After one day, the bleached retinae were mounted onto slides, covered with coverslips and sealed with nail polish. The mounted retinae were then scanned on a flatbed scanner (Epsom Perfection 1640SU) and the images were printed onto 1 mm A4 grid paper – this was simply to obtain a larger outline of the retina onto which the densities of cells could be recorded. The density of cells (per mm^2) was counted at 0.5 mm intervals across the entire surface of each retina, using a 10×10 minigrid eyepiece on a Zeiss Axioscop microscope at ×100 magnification – at this magnification, the

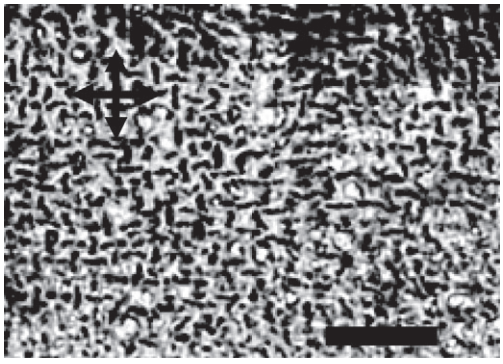

Fig. 2. The retina of *S. lineolata* showing the orthogonal, square, lattice-like arrangement of photoreceptors at a magnification of ×40. The orthogonal PS direction of a local area is shown by the arrows in the top left. Scale bar, 25 μm.

minigrid covered an area of 0.01 mm^2. A starting point was selected on the image and located on the retina using the microscope, and then cell counts at 0.5 mm intervals were taken from this starting point. Results were recorded onto the grid paper. To get the number of cells per mm^2, the number of cells counted in the minigrid was multiplied by a factor of 100. Once all measurements were taken, contour lines were drawn between the points of similar densities (within 5000 cells), and a final map was constructed.

In addition to this, the theoretical sensitivity of the photoreceptors was calculated using an equation by Warrant and Nilsson (Warrant and Nilsson, 1998) that was derived from a similar equation created by Land (Land, 1981). This equation (Eqn 1) uses dimensions of the eye and photoreceptors, and the natural extinction coefficient of the visual pigment, to calculate the sensitivity of the photoreceptors at any point in the retina. The equation is:

$$S = (\pi / 4)^2 A^2 (d / f)^2 [kl / (2.3 + kl)] , \quad (1)$$

where S = sensitivity, A = diameter of the aperture in μm (in this case, the pupil), d = receptor diameter in μm, f = focal length in μm (taken to be 2.3 times the radius of the lens), l = photoreceptor length in μm, and k = 0.01.

Polarized OKR

Three drums were used in this experiment: two controls and one test drum (Fig. 3):

Control Drum 1

This was a black-and-white, vertically striped drum with stripes one inch (2.54 cm) in thickness [a width that has been used previously to elicit visual responses in other cephalopod species (Groeger at al., 2005)], each stripe subtending an angle of 8 deg from the centre of the apparatus. Black stripes were printed onto white paper, which was laminated for waterproofing and, using clear tape, folded around into a drum of diameter 36 cm.

Control Drum 2

This drum was constructed using a sheet of plain linear Polaroid filter of transmission 38% for wavelengths between 400 and 760 nm, with at least 99% transmission efficiency (American Polarizers, Reading, PA, USA). This filter, lined with a layer of white paper that acted as a diffuser for even light distribution, was suitable as it transmits light in a range that is detectable by the coleoid visual system, which peaks in sensitivity to light in the range 470–500 nm. Using clear tape, it was folded around into a drum of diameter 36 cm. The purpose of Control 2 was to ensure that animals were not responding to any imperfections in the test drum that might evoke an OMR during drum rotation, such as the edges of the Polaroid stripes and the joint in the drum.

Test Drum

This drum was constructed using a sheet of 'Polarmotion' (American Polarizers) linear Polaroid filter containing 1-inch thick, alternating, vertical stripes with an e-vector angle set to 0 deg, 45 deg, 90 deg and 135 deg, respectively (American Polarizers), each stripe subtending an angle of 8 deg from the centre of the apparatus. Using clear tape, the sheet was folded around into a drum of diameter 36 cm. This filter was also lined with white paper, which acted as a diffuser for light entering the apparatus from external sources.

The optokinetic measuring apparatus

The apparatus (Fig. 4) was constructed using a clear, plexiglass cylindrical tank (height 34 cm, diameter 30 cm) set atop a stationary disc. Below this sat a rotating disc with a frame mounted to its edge – this frame held each drum in place as it rotated around the tank. This rotating disc was powered by the 12 V motor by means of a natural latex rubber-ring conveyor belt. External illumination (EK-1 Fibre Optic Light, Euromex, Arnhem, The Netherlands) was used to enhance the apparent contrast between the stripes in the drums. A Sony hand-held video camera (DCR-VX 1000E) was mounted onto a tripod and positioned directly above the apparatus to capture the responses of the animals at 25 frames s^{-1}.

A thin layer of sand was placed into the tank to provide a natural-looking, uniformly coloured surface, and a small cylindrical container (height 15 cm, diameter 6.5 cm) was positioned in the middle of the tank to prevent lateral movement. A squid was placed

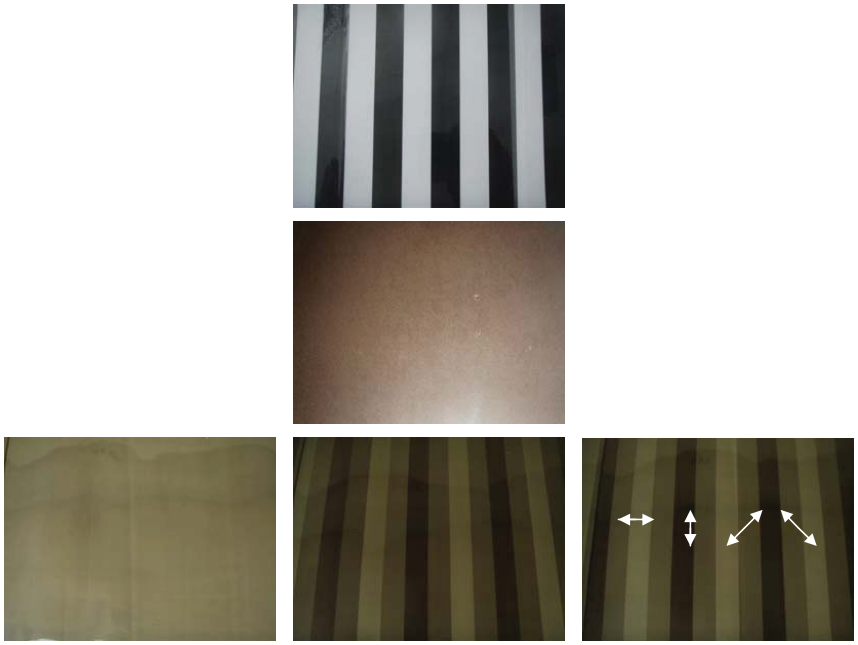

Fig. 3. The three drums used in this study: Control Drum 1 (top), Control Drum 2 (middle) and Test Drum (bottom row). These pictures show the drums as they would appear when looking with non-polarization sensitivity (top, middle and bottom left), looking through a piece of Polaroid filter (orientated vertically – bottom middle, and orientated horizontally – bottom right). The contrast made between adjacent stripes in the test drum can be seen looking through a Polaroid filter. The *e*-vectors produced by each stripe in the test drum are 0 deg, 45 deg, 90 deg and 135 deg.

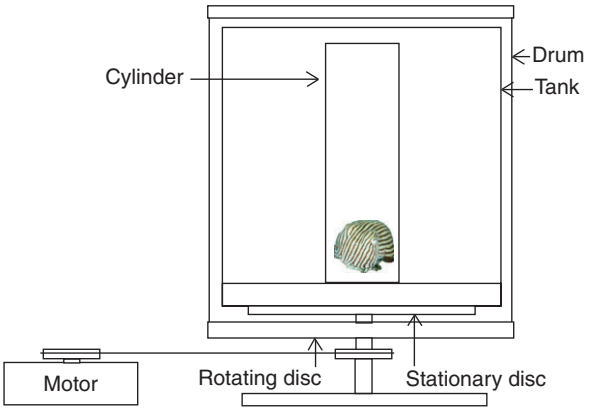

Fig. 4. Schematic diagram of the apparatus used to elicit OKRs in *S. lineolata*. Each specimen was placed into a cylinder inside the tank to prevent them from burying into the substrate.

into the cylinder and allowed to settle for up to 5 min (indicated by cessation of movement). The squid were placed into this centralized container to prevent them from moving out of camera frame and burrowing into the substrate where they were difficult to film. Drums were presented to each animal in random order. Each drum was rotated in one direction for 1 min, then reversed for 1 min, then reversed again for 1 min, at a rotation speed of 12 degrees per second. This protocol was repeated once for each specimen, for each of the three stimuli.

Analysis

Video footage was analysed using the programs Windows Movie Maker (Microsoft) and Adobe Premier Elements (Adobe) frame-by-frame. The angles of the drums were measured on the screen as they rotated using a protractor. The orientation of each eye was also measured individually to determine whether the eyes were moving synchronously and at the same speed, or asynchronously. All measurements were taken using a vertical standard reference line held against the screen, over which the protractor was placed. Measurements of eye movements and drum movement were taken every five frames for up to 12 seconds to show a succession of consistent OKRs in one direction, after which the direction of drum rotation changed to show OKRs in the opposite direction. The eyes of *S. lineolata* are elliptical in shape, and there are straight, black body pattern lines passing over the eye – these lines were used in conjunction with the vertical standard line and the protractor line (which projected out perpendicularly to the line across the eye) to take measurements of eye movement (Fig. 5).

Gain (movement of the eye/movement of the drum in degrees per second) was calculated using the overall average of each OKR slow-phase movement per eye, per animal, for each drum. This value is related to how closely the speed of eye movement was matched to that of the stimulus pattern (Horridge and Sanderman, 1964).

RESULTS

Retinal topography

Investigating the topography of photoreceptors across the retina revealed an increase in cell density in a ventrally shifted horizontal band. Densities peaked at 69,000 cells per mm^2 (Fig. 6). Cells along the dorsal rim of the retinae were unable to be counted owing to tissue damage and folding, but cell numbers in areas approaching the dorsal rim were lower (<42,499 cells per mm^2).

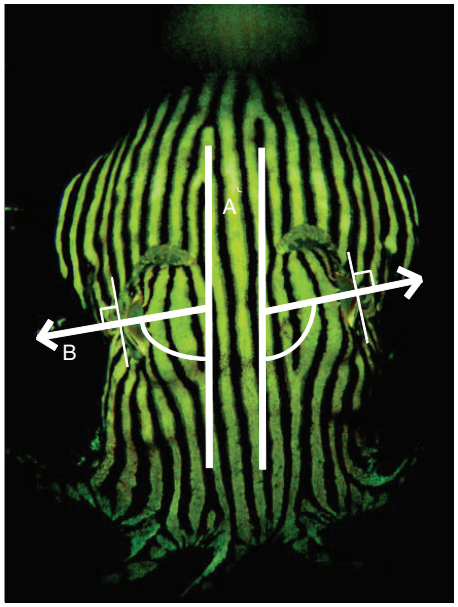

Fig. 5. Each eye of *S. lineolata* was measured individually against a vertical standard reference line (white vertical lines marked 'A'). Using the protractor, a line (marked 'B') was projected from the vertical standard, perpendicular to the black lines that follow the long axis of each eye ball, and through the middle of the pupil/lens, and used to calculate the range of movement in degrees per second.

The following parameters were used to calculate the sensitivity of the eye using Warrant and Nilsson's equation (Warrant and Nilsson, 1998), Eqn 1, where S = sensitivity, $A = 3150\,\mu m$, $d = 7.5\,\mu m$, $f = 4258.5\,\mu m$, $l = 3450\,\mu m$ and k = 0.01. The sensitivity of the eye was calculated to be 11.4.

Polarized optokinesis

Each animal responded to the Test Drum (Fig. 7A) and Control 1 (Fig. 7C) with consistent and strong OKRs. One animal initially responded with an optomotor response (OMR – head and body movements); however this was only short in duration, and the animal

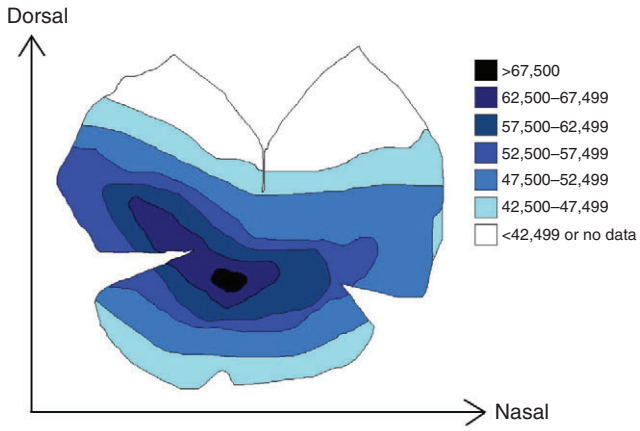

Fig. 6. Ventrally shifted horizontal bands of higher-density photoreceptors were found in the retinae of *S. lineolata*. Numbers are in cells per mm^2.

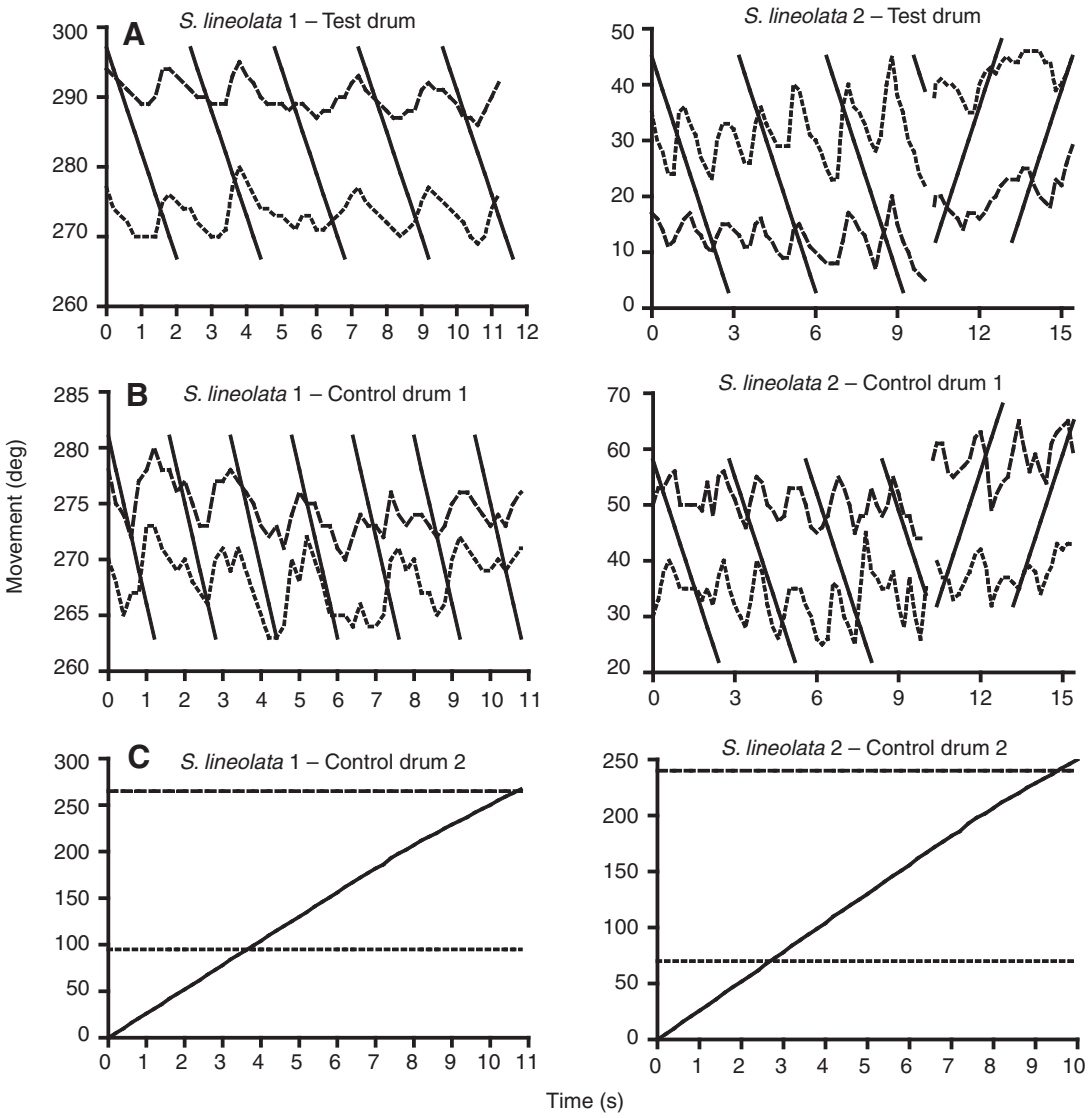

Fig. 7. (A) Both specimens of *S. lineolata* performed consistent OKRs when presented with the Test Drum – a polarizing filter producing alternating e-vectors. The graph of *S. lineolata* 2 shows responses in both directions of drum rotation. The dotted line shows the movement of the left eye, and the dashed line shows the movement of the right eye. The solid line shows the angular speed of the drum. (B) Both specimens of *S. lineolata* performed consistent OKRs when presented with Control Drum 1 – black-and-white vertical stripes. The graph of *S. lineolata* 2 shows responses in both directions of drum rotation. The dotted line shows the movement of the left eye, and the dashed line shows the movement of the right eye. The solid line shows the angular speed of the drum. (C) Neither specimen of *S. lineolata* responded to Control 2 – a plain linear polarizing filter. The dotted line represents the left eye, and the dashed line represents the right eye. The solid line shows the angular speed of the drum.

quickly ceased whole-body movement and switched to performing OKRs within a few seconds. Fig. 7A,C shows the results from both specimens. Responses for *S. lineolata* 2, up until the 10 s mark, were when the drum was rotating in the anticlockwise direction, and clockwise thereafter. In these examples, note that the left eye of the specimen performs slightly larger movements than the right eye in the anti-clockwise direction, and *vice versa* in the clockwise direction.

Consistent OKR sequences often lasted for several seconds, and were performed in both directions, corresponding to the direction of the rotating drum. There were always delays before the onset of performing OKRs after the drums began rotation or changed direction. There was also some slight asynchrony between the movement of the right and left eyes in both animals in response to Control 1 and the Test drum (Fig. 7A,B). One eye always performed larger movements than the other, depending on the direction of stimulus rotation: when the drum was rotated clockwise, the right eye performed over a slightly larger range of motion compared with the left eye; when the drum was rotated anti-clockwise, the left eye performed over a slightly larger range of motion than the right eye. No animals responded with consistent OKRs when presented with Control 2 (Fig. 7B) in either direction, and the eyes of each animal mostly remained completely still.

The gain for each eye of each specimen of *S. lineolata* was calculated for each stimulus (Table 1). These values were calculated using an average of all OKR slow-phase movements displayed in the graphs. Interestingly, the gains for each specimen were higher for the black-and-white control drum compared with the test drum, and *S. lineolata* 2 scored much higher gains than the other specimen in each test.

Table 1. Gain for each eye of both specimens of *S. lineolata* in response to each drum

	S. lineolata 1		*S. lineolata* 2	
	Left eye	Right eye	Left eye	Right eye
Control drum 1	0.58	0.4	0.88	0.94
Control drum 2	0	0	0	0
Test drum	0.46	0.33	0.85	0.65

These values were obtained by averaging each of the slow phases of the OKRs pictured in the graphs.

DISCUSSION

Here, we show that *S. lineolata* possesses an increase in photoreceptor density in a ventrally shifted, horizontal band across the retina, correlating directly with the dorsally shifted, horizontal pupil slit. This strongly implies and supports the suggestion that this bottom-dwelling species, which sits buried in the substrate, leaving just the eyes exposed, possibly requires higher visual acuity in its dorsal visual field, again most likely related to detecting potential predators and prey. Cell density peaked at up to 67,500 cells per mm^2, higher than many of the reef and deep-sea fish species previously studied (Collin and Pettigrew, 1988; Collin et al., 1997), many elasmobranchs (Lisney and Collin, 2008) and even some marine vertebrates such as the harp seal (Mass and Supin, 2003). This strategy results in the animal being more reliant on eye movements to detect and track movement in their visual field while remaining hidden, thus performing OKRs and not OMRs. These features are very similar to those observed in several fish, including the sandlance, which has also evolved a highly developed visual system with independent eye movements to help capture prey occurring in a moving background, while remaining hidden (Pettigrew and Collin, 1995; Fritsches, 1999; Fritsches and Marshall, 2002). Such eye movement is also necessary in other benthic cephalopods, such as many octopus species. It is interesting that *S. lineolata*, as well a just a few others such as the southern dumpling squid, *Euprymna tasmanica*, have adopted this sit-and-wait strategy compared with other coleoids that engage in more active hunting. This is especially unusual given the fact that *S. lineolata* is suspected to be one of the few coleoids that are toxic (Norman, 2000) and might not necessarily need to hide from predators. Thus, the existence of the dorsally shifted pupil might be more related to prey capture than predator avoidance and supports the notion that PS might be used to help detect inconspicuous prey. Their small size and conspicuous body pattern might also have driven *S. lineolata* to adopt a strategy of staying buried or hidden, as apposed to living on or above the substrate, like most other coleoids. Their body-pattern repertoire is much less diverse than in other species – ranging from bold black-and-white stripes (possibly indicating toxicity) to a dark, uniform pattern (Norman, 2000), making camouflage more difficult against many backgrounds.

The sensitivity of the visual system of *S. lineolata* was found to be 11.4 using Warrant and Nilsson's equation (Warrant and Nilsson, 1998). This is quite high for a cephalopod, compared with others that can be as low as 2.6 for the pinhole eye of *Nautilus* and 4.23 for *Octopus* (Land, 1981). Other larger squid such as *Sepioteuthis australis* and *S. lessoniana*, which inhabit a much wider variety of habitats, possess sensitivities of 4.5 and 9, respectively, not significantly different from *S. lineolata*. *Euprymna tasmanica*, a very similarly sized species with similar lifestyle features and habitat preferences, has a slightly lower calculated sensitivity of 4.8 (C.M.T. and J.M., unpublished observations). The sensitivity of human photoreceptors ranges from 0.023 to 32 [for the fovea during broad daylight, to those in the periphery at night (Land, 1981)]. The calculated sensitivity of *S. lineolata* of 11.4 appears quite reasonable for a species that hides during the day and comes out at night, compared with others in a comparative study by Land (Land, 1981), such as other cephalopod and bivalve molluscs and some terrestrial and aquatic arthropods. Although they can remain hidden during the day, they can still hunt, and thus their visual system must be suited to conditions of both light and dark.

Polarized OKR

S. lineolata performed strong OKRs to large-field black-and-white and polarizing contrast stimuli. This is the first evidence of functional PS in response to large-field stimuli in any cephalopod species, using the OKR technique. Other cephalopod species have demonstrated PS using the OMR technique, such as *Sepia plangon*, *S. mestus*, *S. gibba*, *Sepioteuthis lessoniana* and *Idiosepius pygmaeus* (C.M.T. and J.M., unpublished observations). However, interestingly, Darmaillacq and Shashar (Darmaillacq and Shashar, 2008) found no polarized OMR in *Sepia elongata* when using the same technique. This was possibly a result of an interspecies behavioural discrepancy or animal stress, given the fact that *S. elongata* possessed the anatomical components necessary for PS (Darmaillacq and Shashar, 2008). Moody and Parriss (Moody and Parriss, 1961) trained *Octopus vulgaris* to attack certain e-vector stimuli emitted from a torch, whereas Shashar and Cronin (Shashar and Cronin, 1996) trained *O. vulgaris* to select targets displaying a particular e-vector. Moreover, Shashar and colleagues (Shashar et al., 1996) discovered that *Sepia officinalis* produces polarizing patterns in the skin around the eyes, head and arms that are possibly used for intraspecific communication. Further to this, Mäthger and Hanlon (Mäthger and Hanlon, 2006) showed that these polarized signals can be transmitted through expanded chromatophores. Thus, an animal displaying a highly pigmented pattern could still communicate with polarized signals while maintaining crypsis.

There is evidence to suggest that PS might be used for contrast enhancement in the marine environment (Shashar et al., 1998; Shashar et al., 2000; Shashar et al., 2001b). *S. lineolata* not only possesses the structures necessary for PS, but here we demonstrated functional PS using a classic visual response test. While not directly demonstrated here, *S. lineolata* might use PS to break the camouflage of objects in the water column – for example, the transparency of some zooplankton such as the larvae upon which it is known to feed. As far as we could detect, *S. lineolata* does not reflect any polarizing pattern, making polarization communication unlikely in this species. However, without further examination, this possibility, as suggested for other cephalopod species, cannot be ruled out (Shashar et al., 2001a; Shashar et al., 2001b; Boal et al., 2004; Mäthger and Hanlon, 2006).

Polarization sensitivity might help overcome the concealing properties of the caustic flicker of moving shadows cast upon the substrate by surface wave action. The constant stream of shadows cast by waves and ripples might make it difficult to detect items of interest in the water column (particularly items that are transparent, camouflaged by countershading or possess a reflective epidermis), especially in bright, sunny conditions. Thus, PS could increase the chance of detecting an item against this constant flicker, should that item change the polarization of light reflected from its surface.

Optokinetic asynchrony

Interestingly, it appeared that the eyes of both *S. lineolata* were actually moving slightly asynchronously. When the drum was moving clockwise, the right eye made larger optokinetic movements, and *vice versa* when the drum was moving anticlockwise – see Results. This subtle but stronger 'pull' of the eyes in the direction of the moving stimulus seemed to result in the 'leading' eye performing movements over a greater range of motion compared with the 'lagging' eye. The lagging eye did not snap back as far compared with the leading eye – instead it snapped back over smaller distances before resuming the slow-phase movement.

These asynchronous movements are also seen in other animals, such as some fish that are primarily stationary or slow moving

(Fritsches and Marshall, 2002) and the chameleon (Tauber and Atkin, 1976). In these animals, there is a relatively strong sensitivity and response to stimuli that move in the naso-temporal direction. Other animals, however, such as highly mobile fish, show very little sensitivity to stimuli moving in the naso-temporal direction. This illustrates the difference in visual sensitivity to moving stimuli between animals that are primarily stationary and those that are mobile. *S. lineolata*, like the sandlance (Fritsches and Marshall, 2002), is still highly sensitive to naso-temporal stimulation; however, the degree of sensitivity in *S. lineolata* cannot be fully characterized without monocular stimulation tests to determine which direction of movement they are most sensitive to, and to determine the degree of asynchrony between each eye. Further to this, absolute sensitivity to polarized stimuli cannot be measured without conducting tests that vary the velocity of drum rotation and stripe width.

Gain

All gains were below a value of one, indicating that the eyes were driven by the movement of the drum; however, they moved at a slightly slower speed. This is due to 'image slip' on the retina: a slight blur incurred on the retina as the stimulus begins to move, which is detected by the photoreceptors. In order to perceive movement in the visual field, the eyes must compensate for this blur by moving the eyes, and thus this 'image slip' is a necessary process in eliciting a visual response and allowing the subject to detect large-field movement (Horridge and Sandeman, 1964; Carpenter, 1988). Gains up to 0.4 have been obtained in OKR tests on the cuttlefish *Sepia officinalis*, which were held stationary inside a black-and-white rotating drum, also at a rotation speed of 12 degrees per second. Experimentally obtained OKRs always resulted in gains of less than one, and, in some cases, less than 0.2 (Collowijn, 1970). The gains shown here range between 0.33 and 0.94 and show a fairly tight coupling between eye movements and large-field movement. This is possibly due to *S. lineolata* relying more heavily on finely tuned optokinetic eye movements to detect prey, while staying buried (and remaining hidden) in the substrate. The gains for the Test Drum, although noticeably lower than those for the black-and-white Control Drum, were still quite high compared with those obtained for other species such as *Sepia officinalis* (Collewijn, 1970). This result is likely due to some slightly shaky slow-phase responses to the Test Drum in Fig. 7A.

In summary, the data shown here illustrate a concise correlation between several features of the visual ecology of *S. lineolata*. Here, we see that the anatomy of the retina correlates directly with the location and shape of the pupil, to allow for a lifestyle conducted buried in the substrate with a probable emphasis on high visual acuity for the dorsal visual field. Detection of items of interest is probably enhanced owing to the presence of a specialized band of high-density photoreceptors in the retina and to the possession of sensitivity to polarized light based on anatomical and behavioural evidence.

LIST OF ABBREVIATIONS

OKR	optokinetic response
OMR	optomotor response
PS	polarization sensitivity

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