Vision Research 131 (2017) 37–43

Contents lists available at ScienceDirect

Vision Research

journal homepage: www.elsevier.com/locate/visres

Visual sensitivity and spatial resolution of the planktivorous fish, *Atherinomorus forskalii* (Atherinidae; Rüppell, 1838), to a polarized grating

Amit Lerner [a,*], Ron Shmulevitz [a], Howard I. Browman [b], Nadav Shashar [a]

[a] Eilat Campus, Department of Life Sciences, Ben-Gurion University, P.O.B. 653, Beer-Sheva 84105, Israel
[b] Institute of Marine Research, Marine Ecosystem Acoustics Group, Austevoll Research Station, Sauganeset 16, N-5392 Storebø, Norway

ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form 29 November 2016
Accepted 5 December 2016

Keywords:
Optomotor response
Hardyhead silverside
Gilthead seabream
Polarization vision
Planktivory
Acuity
Gill rakers

ABSTRACT

Polarized light detection has been documented in only a small number of fish species. The benefit of polarization vision for fish is not fully understood, nor is the transduction mechanism that underlies it. Past studies proposed that one possible advantage of polarization vision is that it enhances the contrast of zooplankton targets by breaking their transparency. Here, we used an optomotor apparatus to test the responses of the planktivorous Hardyhead silverside fish *Atherinomorus forskalii* (Atherinidae) to vertical unpolarized (intensity) and polarized gratings. We also tested and compared the spatial and temporal resolutions of *A. forskalii* in the intensity and polarization domains. *A. forskalii* responded to the polarization pattern, but only under illumination that included ultraviolet-blue ($\lambda > 380$ nm) wavelengths. The spatial resolution of *A. forskalii* was measured as a minimum separable angle of 0.57° (a 1-mm prey viewed from 100-mm distance). The temporal resolution to unpolarized vs. polarized gratings was constant, at 33 and 10 Hz respectively at most of the stripe widths tested. At the smallest stripe width tested (1 mm = the minimal separable angle), which correlates with the size of prey typically consumed by these fish, the temporal resolution to the polarized grating increased to 42 Hz. We conclude that *A. forskalii* is polarization sensitive, may use polarization vision to improve detection of its planktonic prey, and that polarization may be perceived by the fish via a separate visual pathway than intensity.

1. Introduction

1.1. Polarization vision in the sea

Sea-water is rich in linearly polarized light generated by refraction at the water surface and scattering by the water molecules and suspended hydrosols in the water column (Kattawar, 2013; Lerner, 2014; Lerner, Shashar, & Haspel, 2012). Near the water surface, maximum partial polarization, can be as high as 60% (Sabbah, Lerner, Erlick, & Shashar, 2005; Tonizzo et al., 2009; Voss & Souaidia, 2010) and remains as high as 40% even at depths below 100-m, at least in some viewing directions (Ivanoff & Waterman, 1958, but see lower values in Johnsen, Marshall, & Widder, 2011). Of the >70 aquatic organisms that are known to be sensitive to linearly polarized light, about a dozen are fish, most of them planktivores. These include the rainbow trout, *Oncorhynchus mykiss*; Salmonidae (Hawryshyn & Bolger, 1990; Novales-Flamarique & Browman, 2001), three species of damselfish, *Dascyllus trimaculatus*, *D. melanurus*, and *Chromis viridis*; Pomacentridae (Hawryshyn, Moyer, Allison, Haimberger, & McFarland, 2003; Mussi, Haimberger, & Hawryshyn, 2005), two halfbeak garfish species, *Zenarchopterus dispar* and *Dermogenys pusilla*; Hemiramphidae (Forward, Horch, & Waterman, 1972, Forward & Waterman, 1973, Waterman & Forward , 1970, Waterman & Forward, 1972), and two species of anchovy, *Engraulis mordax*, and *Anchoa mitchilli*, Engraulidae (Fineran & Nicol, 1976; Novales-Flamarique & Harosi, 2002; Novales-Flamarique & Hawryshyn, 1998). Recently, an optomotor response to a polarized grating was reported in post-larvae of the anemone fish *Premnas biaculeatus*, Pomacentridae (Berenshtein et al., 2014). Polarization vision in sea-water has been hypothesized to serve several purposes, such as orientation and navigation (Berenshtein et al., 2014; Lerner, Sabbah, Erlick, & Shashar, 2011), communication and signaling (Boal et al., 2004;

* Corresponding author.
E-mail address: amit.lerner@mail.huji.ac.il (A. Lerner).

http://dx.doi.org/10.1016/j.visres.2016.12.003
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Marshall, Cronin, Shashar, & Land, 1999; Mathger, Shashar, & Hanlon, 2009; Shashar, Rutledge, & Cronin, 1996), and increasing detection distance through contrast enhancement (Novales-Flamarique & Browman, 2001; Sabbah & Shashar, 2006; Shashar, Hagan, Boal, & Hanlon, 2000; Shashar, Hanlon, & Petz, 1998).

1.2. Visual resolution in fish

The spatial resolution (minimum separable angle) of fish ranges between 0.07° and 0.94°, while the temporal resolution (critical flicker fusion frequency; CFF) of fish ranges between 5 and 100 Hz, but in most pelagic species between 20 and 60 Hz, depending on light intensity (Douglas & Hawryshyn, 1990; Sabbah & Hawryshyn, 2013). The temporal resolution of open water pelagic fish such as tuna and swordfish under optimal conditions (warm temperatures, high intensity) is roughly 40 Hz (Fritsches, Brill, & Warrant, 2005). In the polarization domain, information regarding the spatial and temporal resolution of fish is lacking, with the exception of Novales-Flamarique and Browman (2001) study on rainbow trout location (i.e. detection) distance to *Daphnia* against a polarized background. They reported (Fig. 2A therein) a maximum location distance of 60 mm to 0.89 mm prey, which corresponds to a minimum separable angle of ca. 0.85°.

The contradictory evidence about the role of polarization vision in fish, and the rarity of data available, contextualize the objectives of this study, which were to (a) test behaviorally for polarization sensitivity in the planktivorous Red Sea Hardyhead silverside (*Atherinomorus forskalii*; Atherinidae; Rüppell, 1838), and (b) compare its spatial and temporal resolution in the unpolarized and polarized domains. *A. forskalii* is an appropriate model species for this purpose because it is a shallow water pelagic planktivore that inhabits the upper 25 m of the water column, waters rich in polarized light, and visually searches for planktonic prey.

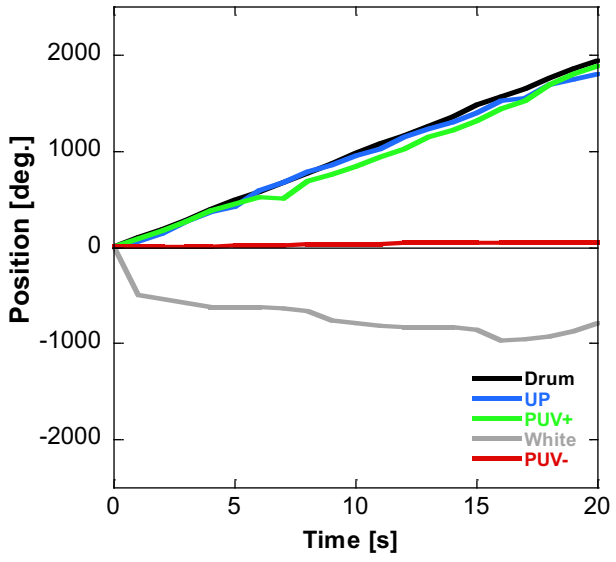

Fig. 1. Fish and drum position during 20 s of drum rotation of *Atherinomorus forskalii*. A positive position represents swimming with the drum rotation direction, while a negative position represents swimming against the direction of the drum rotation. The zero position represents no movement. Lines represent movement of the drum (black) and of the fish in response to unpolarized (UP, blue), white (W, no stripes, blank sheet, grey), and polarized patterns with (PUV+, green) and without (PUV−, red) UV illumination. Angular positions can exceed 360° because the fish could swim more than one circle during the 20 s observation period. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Fish collection and maintenance

Individuals of *Atherinomorus forskalii* (mean ± sd total length = 55.6 ± 0.3 mm, weight = 3.4 ± 0.3 gr, n = 13 fish) were collected from shore and up to 1.5 m depth using a seine which covered an area of 1400 m^2 (for more details see Golani & Lerner, 2007), off the northern tip of the Gulf of Aqaba, Red Sea (N29°33′; E34°57′). The time of day during which *A. forskalii* feeds is poorly known, although it has been observed feeding during both day and night. It is mostly captured during crepuscular periods, when polarization cues are the strongest (Sabbah & Shashar, 2007). Although little is known about its diet, it is a planktivore that feeds on zooplankton. Individuals of the Gilthead seabream *Sparus aurata*; Linnaeus 1758; Sparidae (total length = 28.1 ± 0.3 mm, weight = 5.0 ± 0.2 gr, n = 13 fish), were provided by a local commercial supplier (Ardag Ltd, Eilat). The seabream share a similar shallow benthopelagic habitat with *A. forskalii*. In preliminary experiments, *S. aurata* did not response to a polarized grating. Therefore, it was used as a control to assure that the fish were not responding to any other cue but the polarized grating.

2.2. Optomotor apparatus

An optomotor response (OMR) apparatus, based on a rotating drum, was used to test responses to vertical gratings of different intensity and polarization. The same apparatus was used in previous studies on cuttlefish and fish and is described in detail by Berenshtein et al. (2014), Cartron, Dickel, Shashar, and Darmaillacq (2013), and Darmaillacq and Shashar (2008). Briefly, the method is based on evoking conditioned optomotor responses (body movement) of the fish as it swims with the rotating stripes to stabilize what it sees. Our apparatus included a round drum 39 cm in diameter which is rotated around a stable non-rotating round glass tank 19 cm in diameter filled with sea-water at room temperature (24 °C). Individual fish were placed, one at a time, inside the glass tank during the experiment, and the water was replaced with fresh aerated water between fish. The whole apparatus was placed in a dark chamber in which the only illumination available was from four pairs of UV fluorescent lamps (PHILIPS, ACTINIC BL 15 W, $\lambda > 380$ nm) and four incandescent light bulbs that emitted light in the human visual range and were positioned around the drum. The chamber was ventilated to prevent heating of the water by the incandescent bulbs. The spectral, intensity and polarization characteristics (380–700 nm) projected from the stripes were measured using a custom-made radiometer attached to an optical fiber (USB2000 and UV–VIS 600 μm respectively; Ocean Optics, Dunedin, Florida, USA), also used in a previous study (Lerner et al., 2008). To reduce the acceptance angle, a 5° restrictor was attached to the end of the optical fiber. To measure the polarization, a linear polarizer was placed on the restrictor, and three readings were taken at 0°, 45°, and 90° orientations of the transmitting axis of the polarizer. From these three readings, the partial polarization and the e-vector orientation of the stripes were calculated (for details see Sabbah & Shashar, 2006). The polarized pattern (by Frank Woolley & Co, Reading, PA, USA) that was presented to the fish included repeating sets of four vertical linearly polarized stripes offset by 45° (i.e. 0°, 45°, 90°, and 135° e-vector orientations, transmitting equal intensities). An example of the pattern used can be seen in Darmaillacq and Shashar (2008) (Fig. 2 therin). The stripes transmitted partial polarization between 60% and 85% across the 400–700 nm wavelength range. When UV light was applied, the partial polarization at wavelengths

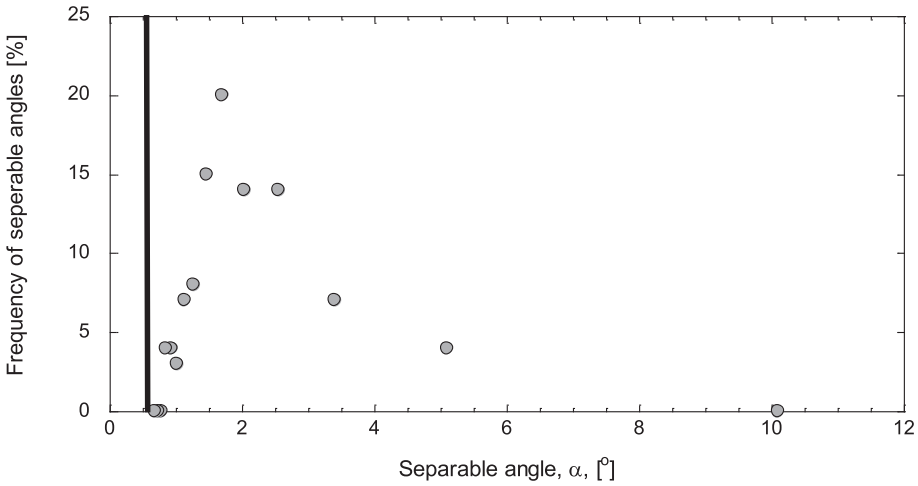

Fig. 2. Frequency distribution of separable angles of rainbow trout (*Oncorhynchus mykiss*) (dots) and *Atherinomorus forskalii* (vertical line) measured when viewing polarized targets. The separable angles of the rainbow trout were calculated using Eq. (2) for location distances extracted from Fig. 2A in Novales-Flamarique and Browman (2001) and a prey size (i.e. target size) of 0.89 mm. Note that the minimal separable angle of the rainbow trout (i.e. maximum spatial resolution) was 0.85°. The vertical black bar represents the minimum separable angle value of 0.57° for *A. forskalii* as measured in this study - its length does not represent percentages.

between 380 and 400 nm increased from 40% to 70%. The contrast of the unpolarized and polarized stripes reached 95% and the calculated contrast between the three polarization oriented stripes followed the values expected by Mallus's law of ca. 1, 1, 1/3. The e-vector orientation, the partial polarization and the unpolarized and polarized contrasts were weakly dependent on wavelength between 400 and 700 nm, and varied by <2°, 5% and 5% respectively within this visual range.

To test for polarization sensitivity, four stimuli were projected to the fish in the optomotor apparatus: polarized vertical stripes of 12.5 mm width, including UV light ($\lambda > 380$ nm; PUV+) and without UV light ($\lambda > 400$ nm; PUV−). As a control, unpolarized black and white stripes (UP; black & white vertical stripes; 12.5 mm width) that were visible to human observers were used to ensure that the fish can see the pattern since, if it does, it is compelled to swim with the rotating grating to stabilize the scenery that it is seeing. Another control pattern, of blanks (no stripes; W), was tested to ensure that there were no artefactual cues produced by the apparatus itself that might evoke responses from the fish. The stripes were illuminated from behind, and the apparatus was covered from above with a black cloth to prevent reflections that might have revealed the polarized stripes to a polarization insensitive visual system. This was confirmed by placing a mirror oriented at 45° to the fish tank while the polarized stripes rotated. The same experiment (including all grating patterns) was also conducted on seabream (*Sparus aurata*).

2.3. Optomotor response (OMR) experiment protocol

The following sequence was applied on each of the 13 fish after placing the fish in the experimental tank for 1 min: (1) 1 min without rotation ('rest'). (2) 1 min of rotation of a randomly chosen stripe pattern and UV illumination (with or without), rotated randomly clockwise (cw) or counterclockwise (ccw). (3) Switching the drum rotation to the opposite direction for another 1 min. (4) Again switching the drum rotation to the original direction applied in stage (2). The stages were repeated for the other patterns and/or UV illuminations. Fish were given a ten second break between changes in drum direction since, in preliminary experiments, this was observed to be sufficient for the fish to come to a complete stop. Rotation speed was set between 0.125 and 0.205 cycles s^{-1} because it generated the best response to the unpolarized stripes in preliminary experiments. In past studies on polarized OMR conducted on cuttlefish, a quarter of a circle was used as the criteria for a positive response (Darmaillacq & Shashar, 2008; Talbot & Marshall, 2010). However, here we applied the more stringent criteria of half a circle, although most fishes examined swam at least one full circle.

2.4. Statistical analysis

Using the OMR test on number of fish, we gave each repeat a yes/no score according to whether the fish responded or not. We tested the null hypothesis of a random response using the Binomial test (with success probability set to 0.5 (50%)). We ran the analysis on 13 fish (replicates; for each of the two species separately), that positively responded to all controls (W, UP). We interpreted the null hypothesis of random response as no response of the fish to the rotating pattern, and the rejection of the null hypothesis as a positive response to the stimulus at the species level.

To quantify how well the fish responded to the rotating polarized pattern, we analyzed a 20 s video sequence of one of the fish that positively responded to the polarized pattern. The distance in degrees that the fish swam from a starting point was recorded every 1 s. We then calculated the fish Gain (the fish angular velocity/the drum angular velocity) to the unpolarized and polarized patterns (Talbot & Marshall, 2010). A Gain of 1 means the fish is swimming with the drum direction and speed, whereas a Gain of 0 means the fish does not swim at all. The fish velocities were estimated from the slope of the linear regression applied to the fish angular distance (in cumulative degrees from a start point) sampled with time during 20 s.

2.5. Temporal and spatial resolution experiment protocol

To measure the critical flicker fusion frequency (CFF; i.e. temporal resolution) of *A. forskalii* against unpolarized and polarized gratings, the rotation speed of the drum (angular velocity) in each test started at 0.8 cycle s^{-1} and was lowered until the fish showed a positive response. This was noted as the maximum angular velocity of the drum to which the fish responded, Ω_{max}. The same experiment was repeated for six different stripe widths: 12.5, 9, 7, 5, 4,

and 1 mm. The CFF [Hz] of the fish was determined by the maximal angular velocity to which the fish responded as calculated by (after Carvalho, Noltie, & Tillitt, 2002):

$$CFF = B \cdot \Omega_{max} = \frac{\pi D}{\lambda} \cdot \Omega_{max} \quad (1)$$

where D is the drum diameter in mm, and the wavelength of the pattern, λ, was taken as the distance between two stripes of the same brightness in the unpolarized pattern or between two stripes of the same e-vector orientation in the polarized pattern, and B is the stripe frequency of the pattern, as determined by λ. Since the number of stripes per cycle in the polarized pattern was twice the number in the unpolarized one (four different stripes vs. two), the maximum frequency resulting from the unpolarized grating was divided by a factor of 2.

The spatial resolution was represented as the separable angle, α, calculated by:

$$\alpha(^\circ) = \arctan\left|\frac{sw}{d}\right| \quad (2)$$

where sw is the stripe width and d is the radial distance from the edge of the fish tank to the rotating pattern (in our case 100 mm), as the fish always swam close to the tank wall (maximal tank radius) when responding to the stimulus. This distance was taken from the fish tank wall and not from its center, as the fish mostly swam in close proximity to the tank wall. α ranged between 0.57° (sw = 1 mm) and 7.12° (sw = 12.5 mm) for both polarized and unpolarized patterns.

2.6. Estimation of prey size by fish gill rakers

As an open water particulate feeding planktivore (A. Lerner, personal observations), the spatial resolution of *A. forskalii* is expected to be related to prey size. To estimate the prey size of *A. forskalii*, the distance between two adjacent gill-rakers was measured from the gills of specimens of varied total lengths caught in the study site and kept in alcohol for collection (The Hebrew University of Jerusalem). The gills were extracted from the fish and the gill rakers were photographed under a stereoscope. The images were analyzed for the distance between gill rakers, using ImageJ software (NIH). Gill-raker density in planktivorous fish (e.g. the European pilchard, *Sardina pilchardus*) decreases with body length according to ae^{-bx} (Costalago & Palomera, 2014; Fig. 3D therein). Therefore, the data here, which included the distance between the gill-rakers, were fit to the inverse of the density equation, which is a growth equation of the form $y = a(1 - e^{-bx})$, where y is the distance between gill rakers and x is the fish total length. From this fit, we estimated the minimum prey size (as assumed by the distance between the gill rakers) of the fish we tested according to their total length. As the gills are the water filtering apparatus of the fish, we assumed that the size of the prey available to this species is larger than the distance between the gill rakers.

The work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

3. Results

3.1. Experiment 1: OMR response to polarized grating

Optomotor responses from 13 fish of each species (*A. forskalii* and *S. aurata*) were measured (Table 1). Both species responded positively to the unpolarized pattern, (>10/13 individuals; Binomial test, $p < 0.001$ for both cw and ccw directions), and randomly responded to the control pattern with no stripes (W; $p > 0.05$). The random response to the control pattern demonstrates that there were no artefactual cues related to the apparatus itself that evoked an optomotor response. *A. forskalii* responded to the polarized grating when UV light was applied (PUV+; $p < 0.05$), but randomly responded in its absence (PUV−, $p > 0.05$). *S. aurata* did not respond to the polarized grating.

During a 120 s movie sequence, *A. forskalii* positively responded (swam with the drum direction) 96 and 93 s against unpolarized (UP) and polarized with UV (PUV+) stimuli, and did not swim at all against the pattern with no stripes (W) and the polarized pattern with no UV (PUV−) illumination (see supplemental movies, S1a and b). Its Gain during 20 s of swimming with the drum direction was 0.96 and 0.95 against the UP and PUV+ respectively (slope = 93.0 and 92.5 °/s, $R^2 = 0.99$, for the UP and PUV+ respectively; Fig. 1).

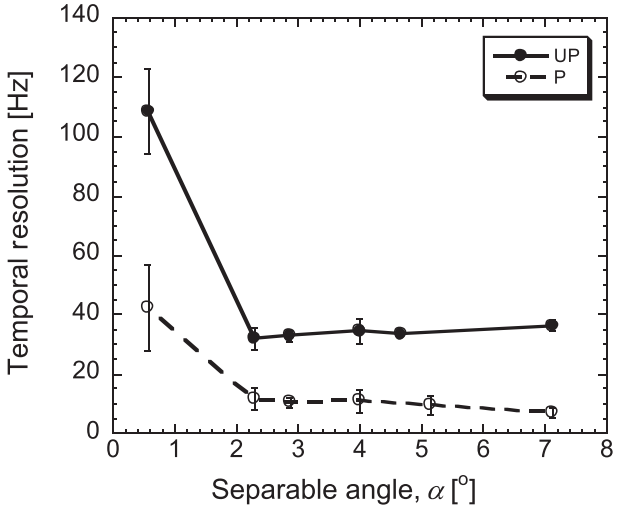

Fig. 3. Resolution (temporal vs. spatial) of the planktivorous fish *Atherinomorus forskalii* (mean ± sd, n = 13) at different stripe widths, presented as the separable angles, α, in which the fish responded to the rotating unpolarized (UP) and polarized (P = PUV+) stripes.

3.2. Experiment 2: spatial and temporal resolution

All *A. forskalii* responded to the minimal stripe width tested of 1 mm (a separable angle $\alpha = 0.57°$), both polarized and unpolarized. Therefore, the actual minimal separable angle of this species could not be determined as it requires providing a value below which the fish do not respond. The separable angle measured here of 0.57° was slightly smaller than the minimum separable angle of 0.85° measured by Novales-Flamarique and Browman (2001) for rainbow trout to a prey under polarized background (Fig. 2).

The mean ± s.d. of the critical flicker fusion frequencies (CFF) that were measured for *A. forskalii* (n = 13 fish) to unpolarized and polarized stripes for $\alpha > 2.29°$ (sw = 3 mm) were 33 ± 2 and 10 ± 2 Hz respectively (Fig. 3). However, for the smallest α value that was examined of 0.57° (sw = 1 mm), the CFF for unpolarized and polarized stripes increased to values of 108 ± 29 and 42 ± 14 Hz respectively. For all stripe widths the drum rotation velocity at which the fish started to respond to polarized stripes Ω_{max} was significantly lower than the Ω_{max} to unpolarized stripes (Paired t-test; $p < 0.001$).

The distance (mean ± se) between gill rakers of the individuals of *A. forskalii* that were tested in the optomotor apparatus was 0.28 ± 0.01 mm (n = 10; total length = 55.6 ± 0.3 mm; Fig. 4).

Table 1
Number of *Atherinomorus forskalii* and *Sparus aurata* that positively responded to the different patterns presented in the optomotor apparatus out of 13 fish tested for each species. The *p*-value represents the significance of a Binomial test of the null hypothesis of a random response (probability = 0.5). cw = drum rotating in the clockwise direction, ccw = drum rotating in the counterclockwise direction. W = white (no stripe) test, UP = unpolarized grating, PUV+ = polarized grating with UV illumination, PUV− = polarized grating with no UV illumination, NS = not significant (*p*-value > 0.05).

Species	W (*p*-value)		UP (*p*-value)		PUV+ (*p*-value)		PUV− (*p*-value)	
	cw	ccw	cw	ccw	cw	ccw	cw	ccw
A. forskalii	2 (NS)	2 (NS)	13 (0.0001)	12 (0.001)	13 (0.0001)	10 (0.03)	4 (NS)	5 (NS)
S. aurata	0 (NS)	0 (NS)	13 (0.0001)	13 (0.0001)	0 (NS)	0 (NS)	0 (NS)	0 (NS)

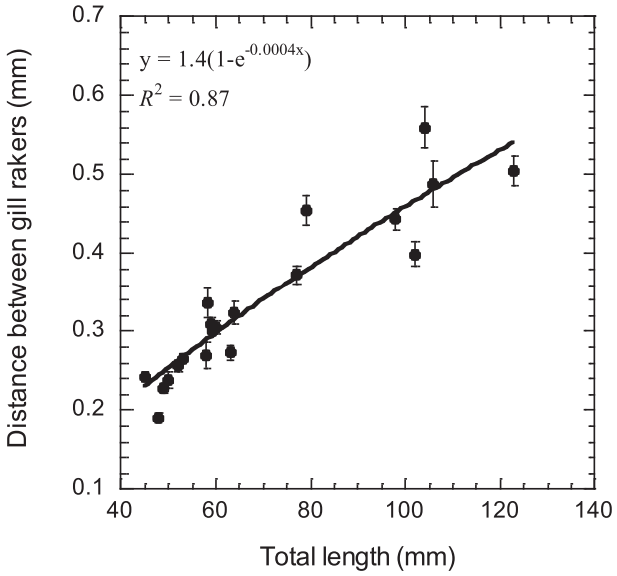

Fig. 4. Mean ± se of the distance between gill rakers and fish total length of *Atherinomorus forskalii*. The data was fitted by the growth equation $y = a \cdot (1 - e^{-bx})$. The distance between the gill rakers is used to estimate the size of prey consumed by the fish since the gill rakers filter the prey. Note that fish size was ca. 0.55 mm, which corresponded to a gill raker distance of ca. 0.28 mm.

4. Discussion

The planktivorous Hardyhead silverside, *Atherinomorus forskalii*, showed a clear OMR to rotating vertical polarized stripes, but only under illumination that included UV light. Thus, *A. forskalii* is only sensitive to polarized light when UV illumination is available. The Gilthead seabream, *Sparus aurata*, showed no OMR to polarized stripes. The first study conducted by Berenshtein et al. (2014), although reported polarization sensitivity in post-larval anemone fish, *Premnas biaculeatus*, did not assess the role of UV light. Thus, this study adds behavioral evidence for the involvement of the UV light in polarization perception in fish, which was demonstrated in electrophysiological studies on salmonids (Hawryshyn, 1992; Hawryshyn, 1997; Novales-Flamarique & Hawryshyn, 1998).

Although the observations reported here beg the question of how polarization is perceived by *A. forskalii*, the current work was intended to assess the contribution of polarization vision to the behavior of this species and not to establish how the signal is transduced by the animal. The state of knowledge about how polarization is perceived by fishes has recently been reviewed (Roberts, 2014).

Negative results of polarization sensitivity (no response to polarization cues) are scarce in the literature. We are aware of only two studies that reported no response to polarized light: the cuttlefish, *Sepia elongata*, and the yellow fever mosquito, *Aedes aegypti* (Bernáth, Horváth, Gál, Fekete, & Meyer-Rochow, 2008; Darmaillacq & Shashar, 2008). Our finding that the Gilthead seabream, *S. aurata*, does not respond to polarized stimuli adds to this small body of information. The insensitivity of *S. aurata* to polarized stimuli suggests differences between the visual systems of the two species studied here. However, since the mechanism of polarization perception in fishes is still unknown (Roberts, 2014), discussing what those differences might be would be highly speculative. This is an important topic for future research.

The CFF of *A. forskalii* was constant at all stripe widths except for an increase at the narrowest stripe width examined (1 mm, for both unpolarized and polarized stripes, corresponding to a separable angle of 0.57°). The CFF for the larger unpolarized stripes matched the value known for open water pelagic predatory fish, ranging between 25 and 65 Hz, with an average at ca. 40–50 Hz (Fritsches et al., 2005; Sabbah & Hawryshyn, 2013). The CFF measured for the polarized grating was lower (10 Hz) than the one measured for the unpolarized grating and matched it only when measured for 1-mm stripe width. This is consistent with a previous study on young cuttlefish (*Sepia officinalis*), in which the CFF measured was 5.5 and 3.3 Hz for the unpolarized and polarized targets respectively (calculated from Cartron et al., 2013). The low CFFs suggest that the resolution to polarized cues requires a different, more complicated and, therefore, slower visual responsivity than to unpolarized cues. For example, if polarization vision requires perception and analysis by more than one photoreceptor, this may lead to slower object detection and lower CFF. This is something that could be tested in future experiments.

In general, the CFF is not expected to change with target size, but to be constant at a given temperature and light intensity. It is reasonable to assume that any observed increase of the CFF with target size is associated with an increase in "visual effort": since CFF is the frequency in time that the target is seen by the observer, a high CFF means that the target is seen in a shorter period of time. Seeing fast (increased number of sampling with time) demands a high performance of the visual system, e.g. high ganglion cell density (which are involved in movement detection), fast transmission of the visual signal throughout the visual system, etc. Thus, the 'visual effort' required from a visual system to see fast moving objects is higher compared to slow moving targets. This is consistent with the need of this species to detect its small transparent and fast-moving prey. Ecological relevance of prey size to a visual predator. *A. forskalii* exhibited a constant CFF at most of the stripe widths tested, both in the intensity and polarization domains, except at 1 mm, which is also close to its minimum prey size. At a stripe width of 1 mm, *A. forskalii* exhibited a ca. 300% increase in CFF compared with the CFF measured at the other stripe widths, in both intensity and polarization domains. We propose that, at this target size, *A. forskalii* increases its visual attentiveness and uses polarization vision to increase the detectability of its planktonic prey; hence, the observed increase in resolution.

In this study, we measured a spatial resolution in the polarization domain of *A. forskalii* as a minimum separable angle of 0.57°.

This value was limited by the minimum polarized stripe width that we could apply of 1 mm and the minimum distance of 100 mm to the target. Therefore, the minimum separable angle of *A. forskalii* in the polarization domain could be lower. However, this value is on the same order of magnitude as the value calculated here from a feeding experiment on the rainbow trout of 0.85° (Fig. 2), and is well within the range of spatial resolution of 0.07–0.94° known for fish (Douglas & Hawryshyn, 1990).

4.1. OMR as a reliable apparatus to test polarization sensitivity

The OMR drum apparatus has been used extensively in vision research. However, a polarized OMR drum apparatus, such as the one used in this study, was first used by Darmaillacq and Shashar (2008), although there was no response from the test animal, and later by Berenshtein et al. (2014) with a positive response from the test animal. A similar apparatus was also used successfully by Talbot and Marshall (2010) and Cartron et al. (2013) on cuttlefish. We contend that this apparatus is reliable for testing polarization sensitivity for the following reasons: (a) any reflections of the stripes that could reveal them to a polarization insensitive system were prevented by illuminating the stripes from behind the pattern and not from above and by covering the apparatus from above with black cloth; (b) the polarized stripes were checked from inside the fish tank using a mirror, and the stripes could not be seen; (c) we used a control fish, the Seabream, which did not respond to the polarized pattern, showing that the fish do not follow any artefactual cue in the polarized pattern; and (d) this exact apparatus was tested successfully (both with negative and positive results) in the above mentioned studies in the past on cuttlefish and fish. Therefore, we conclude that *A. forskalii* did not experience any artefacts that revealed the polarized pattern to it and, therefore, that our conclusion is well-founded.

4.2. Polarization vision as a transparency breaking mechanism and contrast enhancer

Our finding of polarization sensitivity in a shallow water planktivorous fish supports the hypothesis that polarization vision plays a role in planktivory by enhancing prey contrast. To date, the evidence available to test this hypothesis is contradictory. The hypothesis is supported by experiments in the laboratory both of polarization imaging of transparent zooplankton that shows a break in their transparency by up to 92% (Chiou, Place, Caldwell, Marshall, & Cronin, 2012; Johnsen, 2001; Sabbah & Shashar, 2006 and references within), and of behavioral feeding experiments on polarized targets (both biological and non-biological) against polarized backgrounds that show improved prey detection by polarization-sensitive predators (Novales-Flamarique & Browman, 2001; Shashar et al., 1998). Further support comes from the finding by Manor, Polak, Saidel, Goulet, and Shashar (2009) that the prey (*Pontella karachiensis*) itself is repelled from polarized backgrounds, which may be a strategy to avoid predation by polarization-sensitive predators. However, polarization imaging of transparent biological targets at sea suggests that enhanced contrast by polarization is limited under natural conditions, at least in horizontal viewing directions (Johnsen et al., 2011). Therefore, our finding of a behavioral response of the planktivorous fish *A. forskalii* to polarized cues provides additional evidence supporting the role of polarization vision in planktivory.

Acknowledgements

We thank Steve McKuscer and Aiste Klimasauskaite for their technical assistance and for providing and maintaining fish and data collection, and to Arik Diamant for providing us his seine to collect fish. We thank Daniel Golani, the curator of The Hebrew University of Jerusalem fish collection for giving us access to *A. forskalii* specimens. We also thank Ardag Company, Eilat, for supplying fish, and Rafi Fridman and the Underwater Marine Observatory, Eilat, for supplying *Artemia sp.* to feed the fish. We are grateful to the National Center for Marine Agriculture (NCM), Israel Oceanographic and Limnological Research in Eilat for their assistance in keeping the live fish. The project was funded by the Israel Science Foundation (ISF) Grant no. 1081/10 to NS. HIB was funded by the Institute of Marine Research, Norway, project # 81529, "Fine-scale interactions in the plankton".

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.visres.2016.12.003.

References

Berenshtein, I., Kiflawi, M., Shashar, N., Wieler, U., Agiv, H., & Paris, C. B. (2014). Polarized light sensitivity and orientation in coral reef fish post-larvae. *PLoS One, 9*(2), e88468.

Bernáth, B., Horváth, G., Gál, J., Fekete, G., & Meyer-Rochow, V. B. (2008). Polarized light and oviposition site selection in the yellow fever mosquito: No evidence for positive polarotaxis in *Aedes aegypti*. *Vision Research, 48*(13), 1449–1455.

Boal, J. G., Shashar, N., Grable, M. M., Vaughan, K. H., Loew, E. R., & Hanlon, R. T. (2004). Behavioral evidence for intraspecific signaling with achromatic and polarized light by cuttlefish (Mollusca: Cephalopoda). *Behaviour, 141*, 837–861.

Cartron, L., Dickel, L., Shashar, N., & Darmaillacq, A.-S. (2013). Maturation of polarization and luminance contrast sensitivities in cuttlefish (*Sepia officinalis*). *Journal of Experimental Biology, 216*(11), 2039–2045.

Carvalho, P. S., Noltie, D. B., & Tillitt, D. E. (2002). Ontogenetic improvement of visual function in the medaka *Oryzias latipes* based on an optomotor testing system for larval and adult fish. *Animal Behaviour, 64*(1), 1–10.

Chiou, T. H., Place, A. R., Caldwell, R. L., Marshall, N. J., & Cronin, T. W. (2012). A novel function for a carotenoid: Astaxanthin used as a polarizer for visual signalling in a mantis shrimp. *Journal of Experimental Biology, 215*(4), 584–589.

Costalago, D., & Palomera, I. (2014). Feeding of European pilchard (Sardina pilchardus) in the northwestern Mediterranean: From late larvae to adults. *Scientia Marina, 78*(1), 41–54.

Darmaillacq, A.-S., & Shashar, N. (2008). Lack of polarization optomotor response in the cuttlefish *Sepia elongata* (d'Orbigny, 1845). *Physiology & Behavior, 94*(4), 616–620.

Douglas, R. H., & Hawryshyn, C. W. (1990). Behavioural studies of fish vision: An analysis of visual capabilities. In R. H. Douglas & M. B. A. Djamgoz (Eds.), *The visual system of fish* (pp. 526). Cambridge: Chapman and Hall.

Fineran, B. A., & Nicol, J. A. C. (1976). Novel cones in the retina of the anchovy (Anchoa). *Journal of Ultrastructure Research, 54*(2), 296–303.

Forward, R. B., Jr., Horch, K. W., & Waterman, T. H. (1972). Visual orientation at water surface by the teleost *Zenarchopterus*. *Biological Bulletin, 143*(1), 112–126.

Forward, R. B., Jr., & Waterman, T. H. (1973). Evidence for e-vector and light-intensity pattern discrimination by the teleost *Dermogenys*. *Journal of Comparative Physiology, 87*(2), 189–202.

Fritsches, K. A., Brill, R. W., & Warrant, E. J. (2005). Warm eyes provide superior vision in swordfishes. *Current Biology, 15*(1), 55–58.

Golani, D., & Lerner, A. (2007). A long-term study of the sandy shore ichthyofauna in the northern Red Sea (Gulf of Aqaba) with reference to adjacent mariculture activity. *The Raffles Bulletin of Zoology, 14*, 255–264.

Hawryshyn, C. W. (1992). Polarization vision in fish. *American Scientist, 80*(2), 164–175.

Hawryshyn, C. W. (1997). *Ultraviolet-polarization vision: Its role in salmon navigation. Wideband interferometric sensing and imaging polarimetry* (Vol. 3120 (pp. 2–10). San Diego, CA, USA: SPIE.

Hawryshyn, C. W., & Bolger, A. E. (1990). Spatial orientation of trout to partially polarized light. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 167*(5), 691–697.

Hawryshyn, C. W., Moyer, H. D., Allison, W. T., Haimberger, T. J., & McFarland, W. N. (2003). Multidimensional polarization sensitivity in damselfishes. *Journal of Comparative Physiology A – Neuroethology Sensory Neural and Behavioral Physiology, 189*(3), 213–220.

Ivanoff, A., & Waterman, T. H. (1958). Factors, mainly depth and wavelength, affecting the degree of underwater light polarization. *Journal of Marine Research, 16*, 283–307.

Johnsen, S. (2001). Hidden in plain sight: The ecology and physiology of organismal transparency. *Biological Bulletin, 201*(3), 301–318.

Johnsen, S., Marshall, N. J., & Widder, E. A. (2011). Polarization sensitivity as a contrast enhancer in pelagic predators: Lessons from in situ polarization

imaging of transparent zooplankton. *Philosophical Transactions of the Royal Society B-Biological Sciences, 366*(1565), 655–670.
Kattawar, G. W. (2013). Genesis and evolution of polarization of light in the ocean [Invited]. *Applied Optics, 52*(5), 940–948.
Lerner, A. (2014). Underwater polarization by scattering hydrosols. In G. Horváth (Ed.), *Polarized light and polarization vision in animal sciences* (pp. 319–332). Berlin Heidelberg: Springer Verlag.
Lerner, A., Meltser, N., Sapir, N., Erlick, C., Shashar, N., & Broza, M. (2008). Reflected polarization guides chironomid females to oviposition sites. *Journal of Experimental Biology, 211*(22), 3536–3543.
Lerner, A., Sabbah, S., Erlick, C., & Shashar, N. (2011). Navigation by light polarization in clear and turbid waters. *Philosophical Transactions of the Royal Society B: Biological Sciences, 366*(1565), 671–679.
Lerner, A., Shashar, N., & Haspel, C. (2012). Sensitivity study on the effects of hydrosol size and composition on linear polarization in absorbing and nonabsorbing clear and semi-turbid waters. *Journal of the Optical Society of America A, 29*(11), 2394–2405.
Manor, S., Polak, O., Saidel, W. M., Goulet, T. L., & Shashar, N. (2009). Light intensity mediated polarotaxis in *Pontella karachiensis* (Pontellidae, Copepoda). *Vision Research, 49*(19), 2371–2378.
Marshall, J., Cronin, T. W., Shashar, N., & Land, M. (1999). Behavioural evidence for polarisation vision in stomatopods reveals a potential channel for communication. *Current Biology, 9*(14), 755–758.
Mathger, L. M., Shashar, N., & Hanlon, R. T. (2009). Do cephalopods communicate using polarized light reflections from their skin? *Journal of Experimental Biology, 212*(14), 2133–2140.
Mussi, M., Haimberger, T. J., & Hawryshyn, C. W. (2005). Behavioural discrimination of polarized light in the damselfish *Chromis viridis* (family Pomacentridae). *Journal of Experimental Biology, 208*(16), 3037–3046.
Novales-Flamarique, I., & Browman, H. I. (2001). Foraging and prey-search behaviour of small juvenile rainbow trout (*Oncorhynchus mykiss*) under polarized light. *Journal of Experimental Biology, 204*(14), 2415–2422.
Novales-Flamarique, I., & Harosi, F. I. (2002). Visual pigments and dichroism of anchovy cones: A model system for polarization detection. *Visual Neuroscience, 19*(4), 467–473.
Novales-Flamarique, I., & Hawryshyn, C. W. (1998). Photoreceptor types and their relation to the spectral and polarization sensitivities of clupeid fishes. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology, 182*(6), 793–803.
Roberts, N. W. (2014). Polarization vision in fishes. In G. Horvath (Ed.), *Polarized light and polarization vision in animal sciences* (pp. 225–247). Berlin Heidelberg: Springer-Verlag.
Sabbah, S., & Hawryshyn, C. W. (2013). What has driven the evolution of multiple cone classes in visual systems: Object contrast enhancement or light flicker elimination? *BMC Biology, 11*(1), 77.
Sabbah, S., Lerner, A., Erlick, C., & Shashar, N. (2005). Under water polarization vision—A physical examination. In T. R. N. Press (Ed.), *Recent research developments in experimental and theoretical biology* (pp. 123–176). Kerala.
Sabbah, S., & Shashar, N. (2006). Polarization contrast of zooplankton: A model for polarization-based sighting distance. *Vision Research, 46*(4), 444–456.
Sabbah, S., & Shashar, N. (2007). Light polarization under water near sunrise. *Journal of the Optical Society of America A-Optics Image Science and Vision, 24*(7), 2049–2056.
Shashar, N., Hagan, R., Boal, J. G., & Hanlon, R. T. (2000). Cuttlefish use polarization sensitivity in predation on silvery fish. *Vision Research, 40*(1), 71–75.
Shashar, N., Hanlon, R. T., & Petz, A. D. (1998). Polarization vision helps detect transparent prey. *Nature, 393*(6682), 222–223.
Shashar, N., Rutledge, P. S., & Cronin, T. W. (1996). Polarization vision in cuttlefish – A concealed communication channel? *Journal of Experimental Biology, 199*(9), 2077–2084.
Talbot, C. M., & Marshall, J. (2010). Polarization sensitivity in two species of cuttlefish – *Sepia plangon* (Gray 1849) and *Sepia mestus* (Gray 1849) – demonstrated with polarized optomotor stimuli. *Journal of Experimental Biology, 213*(19), 3364–3370.
Tonizzo, A., Zhou, J., Gilerson, A., Twardowski, M. S., Gray, D. J., Arnone, R. A., ... Ahmed, S. A. (2009). Polarized light in coastal waters: Hyperspectral and multiangular analysis. *Optics Express, 17*(7), 5666–5683.
Voss, K. J., & Souaidia, N. (2010). POLRADS: Polarization radiance distribution measurement system. *Optics Express, 18*(19), 19672–19680.
Waterman, T. H., & Forward, R. B. Jr., (1970). Field evidence for polarized light sensitivity in the Fish *Zenarchopterus*. *Nature, 228*(5266), 85–87.
Waterman, T. H., & Forward, R. B. Jr., (1972). Field demonstration of polarotaxis in the fish *Zenarchopterus*. *Journal of Experimental Zoology, 180*(1), 33–54.