The Journal of Experimental Biology 209, 1612-1616
Published by The Company of Biologists 2006
doi:10.1242/jeb.02196

Polarization sensitivity in the red swamp crayfish *Procambarus clarkii* enhances the detection of moving transparent objects

John C. Tuthill[1] and Sönke Johnsen[2,*]

[1]*Biology Department, Swarthmore College, Swarthmore, PA 19081, USA and* [2]*Biology Department, Duke University, Durham, NC 27708, USA*

*Author for correspondence (e-mail: sjohnsen@duke.edu)

Accepted 2 March 2006

Summary

We tested the hypothesis that polarization sensitivity enhances the detection of moving, transparent objects by examining the escape response of the red swamp crayfish (*Procambarus clarkii* Girard) from a visual threat. A transparent, birefringent target trans-illuminated by either partially linear polarized or unpolarized light was advanced toward individual crayfish. The optical axis of the target was aligned such that it would be conspicuous to a viewer with polarization sensitivity when trans-illuminated by polarized light. Under polarized light, significantly more crayfish retreated from the target than under unpolarized light of identical intensity (*P*<0.00005, Fisher's exact test). Whereas the potential for polarization sensitivity has been shown in neurophysiological and structural studies of the visual system of *P. clarkii* and the signal crayfish *Pasifastacus leniusculus* (Dana), our results provide the first behavioral evidence for polarization sensitivity among crayfish. The ecological function of this ability is unclear, but it may enhance the detection of fish with silvered scales, transparent zooplankton or macroinvertebrates. Because escape responses are generally more reliably induced than other behaviors, the method employed in this study may prove useful for examining sensory capabilities in other species.

Key words: polarization vision, polarization sensitivity, crayfish, contrast enhancement, *Procambarus clarkii.*

Introduction

Polarization sensitivity occurs in an eclectic variety of both terrestrial and aquatic species, and serves a variety of functions including orientation (Hawryshyn, 2000; Waterman, 1988), navigation (Goddard and Forward, 1991; Rossel, 1993; Wehner, 1976), intra-specific communication (Cronin and Cronin, 2003), camouflage-breaking (Shashar et al., 1998) and contrast enhancement (Lythgoe and Hemmings, 1967; Shashar and Cronin, 1996). Among invertebrates, the structural and neural basis for polarization sensitivity is particularly well-documented in crustaceans, especially the stomatopods (Marshall, 1988) and crayfish (Glantz, 2001). The ommatidia of many crustacean compound eyes possess adjacent photoreceptors with orthogonally oriented microvilli, establishing the potential for discriminating the orientation of the plane in which the e-vector of light oscillates (Waterman, 1981).

The red swamp crayfish, *Procambarus clarkii*, appears to have all the anatomical and neural structures required to distinguish differences in the dominant e-vector of polarized light. The responses of both the long wavelength-sensitive and short wavelength-sensitive retinular cells are affected by the polarization of the incident light (Waterman and Fernandez, 1970; Muller, 1973; Waterman, 1984). In addition, the lamina and medulla externa of the visual system demonstrate polarization sensitivity in four of the neuronal classes that make up the earliest stages of the visual pathway (Glantz, 1996a; Glantz, 1996b). Inputs from photoreceptors and visual interneurons of *P. clarkii* converge at the medulla externa into two pathways with orthogonal e-vector sensitivity: the sustaining and the dimming fibers (Glantz and McIsaac, 1998). Vertically and horizontally polarized light activate the sustaining and dimming fibers, respectively (Glantz, 1996b). In addition, activation of one pathway inhibits its complement. Antagonistic inputs from these orthogonally oriented polarization analyzers project to both the brain and the medulla terminalis, where polarization opponency between photoreceptors may be integrated (Glantz, 2001). Structural investigations of connectivity patterns between receptors and interneurons have indicated that opponency between these cell groups may provide a mechanism for analyzing and enhancing the sensitivity to the polarization of the signal (Glantz, 1996b). In most of the cells of this pathway, the polarization response is substantially higher for changing e-vector orientation than for fixed orientation, suggesting that polarization discrimination may enhance sensitivity to moving stimuli, a function that is particularly well-developed in crayfish (Glantz, 2001).

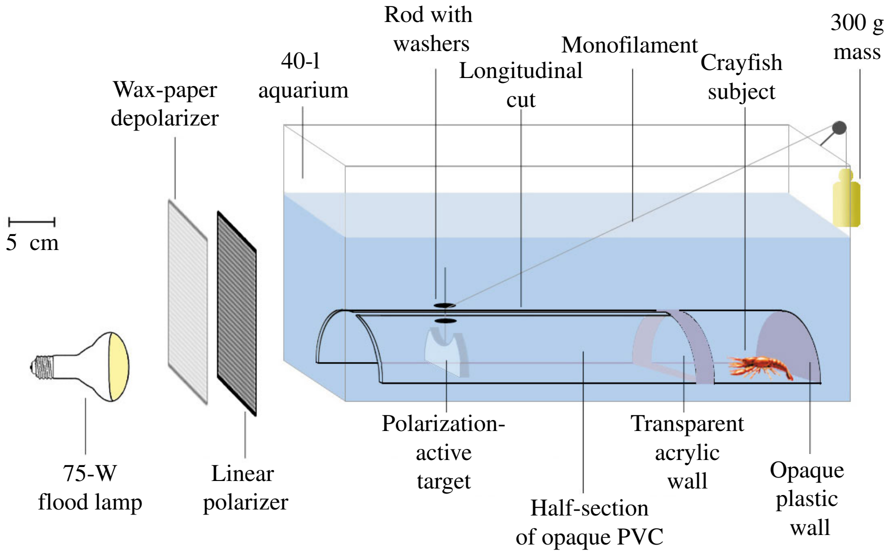

Fig. 1. Schematic diagram of the experimental apparatus used in the study. The PVC half-section of pipe is transparent in this figure to show the inside of the device. In the actual setup the polarizer/diffuser sheets were sandwiched together against the side of the tank.

In this study we test whether the neural evidence for polarization sensitivity in *P. clarkii* translates into a behavioral response. In particular, we test whether polarization information in an otherwise cryptic moving stimulus increases the ability of *P. clarkii* to detect it.

Materials and methods

Male and female red swamp crayfish, *Procambarus clarkii* (Girard) (6–9 cm length) were bought from a biological supply company (Carolina Biological Inc., NC, USA). They were maintained in two aerated and filtered 120 l aquaria on a 12 h:12 h light:dark cycle at 20°C, and fed peas weekly in separate 40 l aquaria.

The experimental apparatus consisted of a half-section of white plastic pipe (10 cm diameter, 48 cm length) that was partitioned into two chambers separated by a sheet of acrylic (Fig. 1). The 13 cm long rear section, where each crayfish was isolated, had a second wall of opaque white plastic. A longitudinal cut (33 cm length) was made down the top of the front section of the pipe, along which a transparent target could be advanced toward the crayfish chamber. The target was attached *via* a short rod and monofilament to a 300 g mass. When dropped, the mass pulled the target down the length of pipe at an average velocity of 0.3 m s^{-1} over 0.65 s. The cut ended 2 cm before the crayfish chamber, thus stopping the target before it struck the chamber wall.

The transparent target was made of a sandwich of 0.3 cm thick clear acrylic and an optically anisotropic, colorless sheet of Mylar (Dura-lar Clear Overlay Film, Medium Weight 0.003; Graffix, Cleveland, OH, USA). The Mylar was aligned so that it converted the linearly polarized light to nearly circularly polarized light. Thus, while inconspicuous under transmitted unpolarized light, the target's optical properties

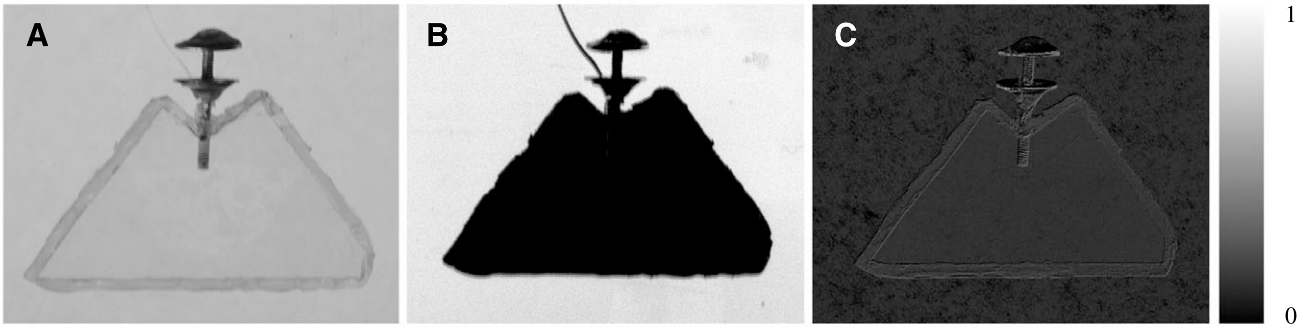

Fig. 2. The target was constructed of clear acrylic covered with colorless, polarization-active Mylar. (A) Unmodified photograph of the transparent target viewed under polarized light. (B) Polarization contrast image of the target (trans-illuminated by vertically polarized light) generated by taking two photographs through a polarizing filter that was rotated by 90° between exposures. Each pixel brightness is equal to $255[(I_v-I_h)/(I_v+I_h)]$, where I_v and I_h are the pixel values when the transilluminated target is viewed through a vertical and horizontal polarizer respectively. (C) Same as in B but with wax-paper diffuser depolarizing the light. Because the neural responses of *P. clarkii* to changing e-vector depend on the rate of change (Glantz, 2001), and possibly on color, it is impossible to simulate how the moving target appears under each condition. Thus B and C are probably only an approximate indication of the contrast of the target under polarizing and non-polarizing conditions, respectively. The bar on right shows the contrast scale.

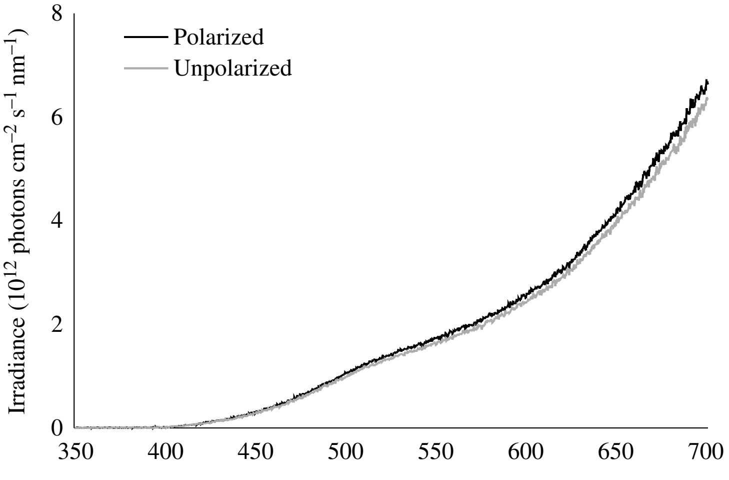

Fig. 3. Spectral irradiance inside the crayfish chamber under both polarization conditions. The irradiance probe faced the light source.

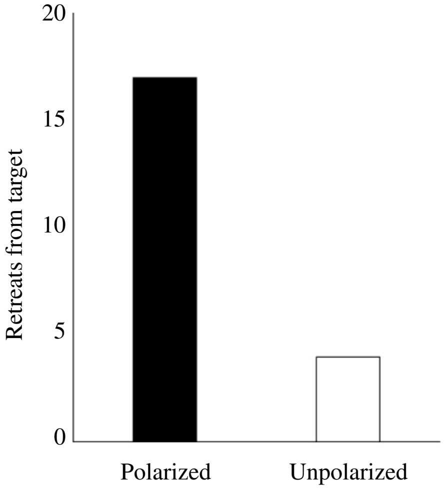

Fig. 4. Response of *Procambarus clarkii* to the approach of the target under polarized and unpolarized conditions (*N*=20 for each treatment). Crayfish retreated from the target significantly more often in the polarized condition (*P*<0.00005).

rendered it highly visible when viewed under transmitted polarized light by a viewer with polarization sensitivity (Fig. 2).

The testing apparatus rested on a sheet of acrylic within a 40 l aquarium filled with water. A 75 W flood lamp was mounted 10 cm from the front of the tank. The light passed through a sandwich of a linear polarizer (HN38S, Polaroid Co., Waltham, MA, USA) and a wax-paper diffuser/depolarizer. For the polarized light trials, the polarizer followed the depolarizer. For the unpolarized light trials, the polarizer preceded the depolarizer. Thus, the two lighting conditions differed only in polarization and not in intensity or spectral distribution (Fig. 3).

Experiments were conducted in June and July of 2005, between 10:00 and 16:00 DST. Before each day's trials, the experimental tank was rinsed and filled to approximately 20 cm depth with deionized water. Each trial then proceeded as follows. A crayfish was placed in the experimental chamber and allowed a 5-min acclimation period. If, after 5 min, the crayfish did not face toward the light source, an extra period of up to 5 min was allowed for the crayfish to orient correctly. After 10 min, whether the crayfish was oriented towards the light or not (5 out of 40 did not), the target was advanced by dropping the mass from the top of the aquarium. Each animal was tested once under either the polarized or unpolarized condition.

The responses of the crayfish to the advancing target were recorded through the glass bottom of the aquarium using a digital video camera. The presence or absence of a response was recorded by a blind (ignorant of light condition) examination of the video recordings of each experimental trial. Based on preliminary observations prior to testing, a positive response was defined as a retreat by the crayfish of >2 cm. No animal retreated before the target moved.

Results

Under partially linearly polarized light, *P. clarkii* was four times as likely to retreat from an advancing, transparent, polarization-active object as under unpolarized conditions (*P*<0.00005, Fisher exact test; Fig. 4). Out of 20 trials that took place under un-polarized light, four of the subjects retreated from the stimulus. Under polarized light, 17 out of 20 crayfish retreated. The retreat response generally consisted of the crayfish pushing off with the walking legs and chelipeds, and occasionally of a tail-flip escape response. Tail-flip escapes were observed only under polarized light conditions, in four out of the 20 trials.

Discussion

The highly significant difference in response between treatments suggests that crayfish were more aware of the advancing target under polarized light conditions. These results provide the first behavioral demonstration of polarization sensitivity in crayfish and one of the few studies that show contrast enhancement.

Whether the increased number of retreats is due to enhanced detection of the transparent target itself, or enhanced detection of its motion (or both), is uncertain. *P. clarkii* are sensitive to moving visual stimuli over a velocity range of at least four orders of magnitude (Glantz, 2001), indicating that motion detection is a critical component of the crayfish visual system. A significant portion of the primary visual synapse (Glantz and Bartels, 1994) and the ascending optic tract (Wiersma and Yamaguchi, 1966) are devoted to motion sensitivity. Glantz (Glantz, 2001) proposed that the polarization sensitive-neurons might also contribute to this pathway. This hypothesis is supported by the existence of tangential cells in the crayfish optic lobe that demonstrate multidimensional selectivity to contrast, motion and e-vector, and enhanced responses to changes in e-vector orientation *versus* responses to fixed orientations (Glantz, 1996b; Glantz, 2001). Detection of movement using polarization opponency is analogous to using color opponency to detect movement in the absence of intensity contrast (Bernard and Wehner, 1977). The results of this study are consistent with, but do not confirm, the hypothesis that crayfish use such a polarization-opponency mechanism to enhance motion detection.

Ecological function of polarization sensitivity

The ecological function of polarization sensitivity in this species is unclear. *P. clarkii* is an abundant opportunistic omnivore found in seasonally flooded wetlands, lakes and streams throughout North America, often concealing itself in burrows or under rocks and logs (Gherardi, 2002). At shallow depths in streams or lakes, the overhead polarization pattern is essentially a distortion of the polarization pattern of skylight (Horváth and Varjú, 2004). At angles more than 48° from the vertical, this pattern is replaced by a complex pattern due to reflections from underwater objects and scattering of downwelling light (Horváth and Varjú, 2004). With increasing turbidity, the polarization vanishes.

Transparent prey (e.g. zooplankton, macroinvertebrates) viewed against polarized backgrounds may be detectable because the scattering of light within their tissues can rotate or disrupt the background polarization pattern (Shashar et al., 1998). Fish, the chief predators of crayfish (Nyström, 2002), may be detected *via* the polarized reflections from their mirror-like scales (Shashar et al., 2000). Analysis of reflected polarized light may also allow crayfish to detect and recognize substrata, plants, and other underwater features (Novales Flamarique and Hawryshyn, 1997). Levels of polarization in the upper photic zone are highest during crepuscular periods (Novales Flamarique and Hawryshyn, 1997) when crayfish are most active (Gherardi, 2002), indicating that crayfish forage when they are most visually prepared to detect prey and avoid potential predators.

Understanding the function of polarization sensitivity in *P. clarkii* is complicated by the fact that the polarization information may interact with color vision. An ommatidium of *P. clarkii* has seven photoreceptors containing a visual pigment that peaks at 530 or 567 nm (depending on chromophore) (Zeiger and Goldsmith, 1989). With the exception of those in the dorsal retina, the ommatidia also contain an eighth cell expressing a visual pigment that peaks at 440 nm (Cummins and Goldsmith, 1981). Although sample sizes are low, it appears that, in the anterior ommatidia, most of the 440 nm receptors are sensitive to horizontal polarization, and most of the 530/567 nm receptors are sensitive to vertical polarization. In the dorsal retina, which contains only the 530/567 nm receptors, most of the cells are sensitive to vertical polarization (Waterman and Fernandez, 1970). These results suggest both regional and chromatic specialization of polarization sensitivity and complicate any attempt to understand its function.

Escape responses as indicators of sensory abilities

One of the difficulties in research on polarization sensitivity is that the behavioral responses have generally been marginal (reviewed by Waterman, 1981). Thus, many significant results have been based on small differences and large samples sizes. This makes experiments difficult to replicate and generates concerns about potential biasing artifacts. In contrast, this study achieved a P value less than 0.00005 with a sample size of only 40 animals. We attribute the strength of these results to the stereotypical and critical nature of escape responses. Whereas feeding, orientation, and social behaviors are important to the survival and fitness of an organism, they tend to be less reliably induced than escape responses, particularly after a period of captivity. For this reason, we believe that sensory assays based on escape responses may be quite useful for other species and other sensory abilities.

We thank Rachel Merz for a critical reading of the paper. The Norman A. Meinkoth Award at Swarthmore College provided funding for J.T. S.J. was supported in part by a grant from the National Science Foundation (IOB-0444674).

References

Bernard, G. D. and Wehner, R. (1977). Functional similarities between polarization vision and color vision. *Vision Res.* **17**, 1019-1028.

Cronin, T. W., Shashar, N., Caldwell, R. L., Marshall, J., Cheroske, A. G. and Chiou, T. H. (2003). Polarization vision and its role in biological signaling. *Integr. Comp. Biol.* **43**, 549-558.

Cummins, D. and Goldsmith, T. H. (1981). Cellular identification of the violet receptor in the crayfish eye. *J. Comp. Physiol. A* **142**, 199-202.

Gherardi, F. (2002). Behaviour. In *Biology of Freshwater Crayfish* (ed. D. M. Holdich), pp. 258-290. Ames, IA: Iowa State University Press.

Glantz, R. M. (1996a). Polarization sensitivity in crayfish lamina monopolar neurons. *J. Comp. Physiol. A* **178**, 413-425.

Glantz, R. M. (1996b). Polarization sensitivity in the crayfish optic lobe: Peripheral contributions to opponency and directionally selective motion detection. *J. Neurophysiol.* **76**, 3404-3414.

Glantz, R. M. (2001). Polarization analysis in the crayfish visual system. *J. Exp. Biol.* **204**, 2383-2390.

Glantz, R. M. and Bartels, A. (1994). The spatiotemporal transfer function of crayfish lamina monopolar neurons. *J. Neurophysiol.* **71**, 2168-2182.

Glantz, R. M. and McIsaac, A. (1998). Two-channel polarization analyzer in the sustaining fiber dimming fiber ensemble of crayfish visual system. *J. Neurophysiol.* **80**, 2571-2583.

Goddard, S. M. and Forward, R. B. J. (1991). The role of the underwater polarized light pattern, in sun compass navigation of the grass shrimp, *Palaemonetes vulgaris*. *J. Comp. Physiol. A* **169**, 479-491.

Hawryshyn, C. W. (2000). Ultraviolet polarization vision in fishes: possible mechanisms for coding e-vector. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **355**, 1187-1190.

Horváth, G. and Varjú, D. (2004). *Polarized Light in Animal Vision: Polarization Patterns in Nature*. Berlin, New York: Springer.

Lythgoe, J. N. and Hemmings, C. C. (1967). Polarized light and underwater vision. *Nature* **213**, 893-894.

Marshall, N. J. (1988). A unique colour and polarization vision system in mantis shrimps. *Nature* **333**, 557-560.

Muller, K. J. (1973). Photoreceptors in the crayfish compound eye: electrical interactions between cells as related to polarized light sensitivity. *J. Physiol. Lond.* **232**, 573-595.

Novales Flamarique, I. and Hawryshyn, C. W. (1997). Is the use of underwater polarized light by fish restricted to crepuscular time periods? *Vision Res.* **37**, 975-989.

Nyström, P. (2002). Ecology. In *Biology of Freshwater Crayfish* (ed. D. M. Holdich), pp. 192-235. Ames, IA: Iowa State University Press.

Rossel, S. (1993). Navigation by bees using polarized skylight. *Comp. Biochem. Physiol.* **104A**, 695-708.

Shashar, N. and Cronin, T. (1996). Polarization contrast vision in octopus. *J. Exp. Biol.* **199**, 999-1004.

Shashar, N., Hanlon, R. T. and Petz, A. D. (1998). Polarization vision helps detect transparent prey. *Nature* **393**, 222-223.

Shashar, N., Hagan, R., Boal, J. G. and Hanlon, R. T. (2000). Cuttlefish use polarization sensitivity in predation on silvery fish. *Vision Res.* **40**, 71-75.

Waterman, T. H. (1981). Polarization sensitivity. In *Handbook of Sensory Physiology*, *Vol. 7/6B* (ed. H. Autrum), pp. 281-469. New York: Springer-Verlag.

Waterman, T. H. (1984). Natural polarized light and vision. In *Photoreception and Vision in Invertebrates* (ed. M. A. Ali), pp. 63-114. New York: Plenum Press.

Waterman, T. H. (1988). Polarization of marine light fields and animal orientation. *SPIE* **925**, 431-437.

Waterman, T. H. and Fernandez, H. R. (1970). E-vector and wavelength discrimination by retinular cells of the crayfish *Procambarus*. *Z. Vergl. Physiol.* **68**, 154-174.

Wehner, R. (1976). Polarized-light navigation by insects. *Sci. Am.* **235**, 106-115.

Wiersma, C. A. and Yamaguchi, T. (1966). The neuronal components of the optic nerve of the crayfish as studied by single unit analysis. *J. Comp. Neurol.* **128**, 333-358.

Zeiger, J. and Goldsmith, T. H. (1989). Spectral properties of porphyropsin from an invertebrate. *Vision Res.* **29**, 519-527.